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FERTILIZER REQUIREMENTS OF COFFEE GROWN ON CATALINA CLAY IN PUERTO RICO

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INTRODUCTION

Coffee is the second most important crop in Puerto Rico. About twenty per cent of the population obtain their livelihood from it. Yet, the coffee production per unit of area is very low: less than two hundred pounds of marketable coffee per acre.

Among the more obvious reasons for the low production per acre of Puerto Rican coffee, one should note that the local variety grown of *Coffea arabica* has a relatively low yield, although its berries produce a quality liquor of excellent aroma and taste, greatly preferred in the European and Cuban market. Added to the loss of this specialized market is the heavy damage inflicted by recurrent hurricanes, which has prevented the proper renovation of the coffee and shade trees and the adoption of somewhat more costly but more effective methods of cultivation. The main portion of the coffee area is on soils of the Catalina, Alonso, Los Guineos and Cialitos series, which are acid and quite low in their base exchange capacity. Because of lack of adequate fertilizer experiments on these soils, coffee growers do not know the most effective and desirable fertilizer applications to obtain maximum yields of this crop.

In his bulletin on fertilizers for coffee, McClelland (2) presented the results obtained in a series of fertilizer studies with coffee carried out at the Federal Agricultural Experiment Station at Mayaguez and elsewhere on the Island. In the summary, he made the following statements:

1. "The production over an 8-year period showed that potash was effective in increasing yield, and that this was true particularly where nitrogen was used in addition to potash." (2, p. 32.)
2. "Growth and yield failed to show that the addition of phosphoric acid was of benefit." (2, p. 32.)
3. "Until further evidence is obtained on this point, it is believed that a fertilizer for coffee should run proportionally high in potash, such, for example, as one obtained by mixing ammonium sulphate and potassium sulphate in equal parts by weight and containing approximately 10 per cent nitrogen and 24 per cent potash." (2, p. 33.)

In order to obtain more information relative to the fertilizer requirements for coffee in Puerto Rico, a fertilizer test was started by Mr. Vicente Medina on the farm of Mr. Juan Esteva, at Lares, on January 12, 1932. This experiment was carried out as originally outlined until the seventh crop was harvested in 1939, when, as a result of a study of the yield data of the first six crops, it was decided to alter the procedure in use up to that time in the check plots. Thus, the plots that had received fertilizer applications for the first seven crops, were treated similarly for the eighth and ninth crops, the last ones of this experiment; but for these last two crops, fertilizer applications were given to some of the check plots which had received no fertilizer applications for the first seven crops.

As a result of the study of the first six crops of this experiment, another fertilizer test with coffee was started at Mayaguez. Only three crops were harvested in this experiment due to the sale of the farm on which the experiment was established, and that the new owner needed the land for other purposes.

A description of the treatments tested in these experiments and the results obtained follows in detail.

EXPERIMENT ON "CATALINA CLAY" AT LARES

Table 1 presents the treatments tested and the mean yields obtained in the plots that received the same fertilizer applications for the nine crops of the experiment established at Lares, on "Catalina Clay." In this experiment, the plots consisted of ten trees each, planted at a distance of eight feet between adjacent trees. Each plot was, therefore, 640 square feet in area, or approximately $\frac{1}{68}$ of an acre. The plots were arranged in a randomized block layout with ten replications. Five check plots, receiving no fertilizer applications, were included in each block of plots of the experiment. In table 1, however, the yields of those check plots that continued as such until the end of the test are the only ones presented.

In 1939, as has already been mentioned in the *Introduction*, a statistical study of the results obtained in the first six crops of this experiment was

made. As a result of that study, the following conclusions (1) were derived:

"The fact that no difference between the mean yields of treatments 'D', 'I' and 'J' exceeds the critical value for significance at the five per cent point, shows that potash applied in excess of 15 units did not increase significantly the yields, when the crop received in addition only five units

TABLE 1

Mean yields in hundredweights market coffee per acre of the fertilizer test performed at Lares for the nine crop cycle: 1933–1941

TREATMENTS				REPLICATIONS										
Letter	NH_3	P_2O_5	K_2O	1	2	3	4	5	6	7	8	9	10	Total
	units	*units*	*units*											
A	5	5	15	2.47	4.23	3.60	3.68	3.51	4.73	4.62	3.00	5.89	3.91	39.64
B	10	5	15	3.56	3.61	3.22	3.61	4.03	5.66	4.60	5.55	5.47	3.76	43.07
C	15	5	15	4.03	5.09	3.25	4.75	5.18	4.75	4.75	6.47	6.13	4.05	48.45
D	5	0	15	2.89	4.84	3.27	2.92	4.53	3.89	3.56	3.56	3.30	3.42	36.18
E	5	10	15	3.14	4.02	2.84	3.78	3.38	4.07	6.62	6.00	2.76	4.43	41.04
F	5	15	15	3.16	3.51	3.65	3.75	5.35	5.65	5.36	4.67	*4.82*	5.06	44.98
G	5	5	20	4.03	2.97	4.10	2.70	4.54	4.57	3.57	5.19	*4.32*	4.00	39.99
H	5	5	25	4.89	2.92	3.05	3.55	5.19	3.44	3.91	4.89	4.89	4.73	41.46
I	5	0	20	3.80	3.74	3.28	3.65	5.60	4.72	2.82	5.18	4.10	4.07	40.96
J	5	0	25	4.65	2.59	2.76	2.81	5.48	3.80	4.68	3.12	4.19	5.47	39.55
K	0	0	0	3.41	2.54	2.42	2.38	3.80	2.90	3.31	4.22	2.23	*3.17*	30.38
Total				40.03	40.06	35.44	37.58	50.59	48.18	47.80	51.85	48.10	46.07	445.70

Mean

(8) 3.964	(7) 3.999
(3) 4.307	(4) 4.146
(1) 4.845	(6) 4.096
(10) 3.618	(9) 3.955
(5) 4.104	(11) 3.038
(12) 4.498	
	44.570

Notes: 1 unit = 7.5 pounds of substance per acre. Values in italics are estimated.

of nitrogen. A similar conclusion is derived on comparing the mean yields of treatments 'A', 'G' and 'H', when the crop received five units each of nitrogen and phosphoric acid. Therefore, on this basis, it can be concluded that applications of potash at rates higher than the minimum 15 units used in this experiment were not effective in increasing the yields over the yield produced by the minimum 15 units of potash used.

"Neither five nor 10 units of phosphoric acid were sufficient to increase significantly the yields, when the crop received in addition five units of nitrogen and 15 units of potash. This conclusion is based on a comparison of the mean yields of treatments 'D', 'A', and 'E'. The application of 15 units of phosphoric acid in treatment 'F', however, produced a significant increase in yield when compared with the no-phosphoric acid application of treatment 'D'.

"Neither 10 nor 15 units of nitrogen produced any significant increases in yield over that produced by five units of nitrogen, when the crop received in addition five units of phosphoric acid and 15 units of potash. This is deduced from a comparison of the mean yields of treatment 'A', 'B', and 'C'.

"The tendency of the yields, however, is to increase with increasing amounts of both nitrogen and phosphoric acid. This is in full accord with the idea that the yield of a crop depends on the concentration of nutrients in the soil, and that the more nutrients, the higher the yield up to an optimum point. A small application of some nutrient may not prove its effectiveness in increasing the yield due to the heterogeneity of the soil, while a larger amount of the same nutrient may prove effective in so doing. This behaviour cannot be interpreted as indicating that the small application has no effect while the large application has a real effect. On the contrary, both applications are effective, only that the small one is not sufficiently effective to influence the yield statistically under the conditions in which the experiment is performed. This has happened in this case with the applications of phosphoric acid where the application of both five and 10 units did not produce a statistical increase in yield, while the 15-unit application did produce it.

"The applications of nitrogen were, according to the statistical analysis, not significant, but when used in conjunction with the application of five units of phosphoric acid—which had not demonstrated any significant effect by themselves—produced significant increases in yield.

"The applications of potash, beyond the minimum 15 units used, however, have produced no significant increases. This is not to be interpreted in the sense that potash is not necessary for high coffee yields, but in the sense that the nutrient requirements of the crop had been fulfilled with an amount of potash which was no larger, and may have been smaller, than the soil content plus the 15 units applied as minimum.

"If when the project is closed, the results are the same as those obtained to date, the recommendation would have to be in favor of the use of a fertilizer analysis containing the maximum amounts of nitrogen and phosphoric acid and the minimum amount of potash used in this test, that is, an application of about 112.5 pounds per acre each of ammonia, phosphoric

acid and potash. At that time there would be no evidence on which to recommend the use of a smaller amount of potash, since this range of potash application has not been investigated."

It must be pointed out that the use of the small amounts of phosphoric acid and the large amounts of potash tested in this experiment were due to the results obtained by McClelland (2) mentioned above. The results at the date of this study indicated, however, trends altogether different from those obtained by McClelland (2), and, accordingly, from the results expected at the time that the experiment was started.

The statistical analysis of the yield data obtained in the whole nine-crop cycle appears in table 2, and the results of the evaluation of the statistical significance of the yield differences that may be attributed to differences in the rates of application of the fertilizer substances are presented

TABLE 2

Analysis of the total sum of squared deviations of the data of table 1

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Blocks	9	27.3257		
Treatments	10	21.4278	2.1428	3.30**
Error	87	56.5363	0.6498	
Total	106	105.2898		

There are highly significant differences between the treatment means.
Values to be exceeded for significance between two 10-plot means:
At the 5% point..... 0.717 hundredweights market coffee per acre
At the 1% point..... 0.950 hundredweights market coffee per acre

in table 3. This table indicates that the conclusions to be derived from the results of the whole nine-crop cycle are about the same as were derived from the interpretation of the results of the first six crops of the experiment. The effect of the application of the 15 units of the nitrogen has now proved to be significant: a conclusion that was suggested but not verified by the previous study.

The lack of response to applications of potash in excess of the minimum application of 15 units, or 112.5 pounds of potash per acre, suggested the possibility of maintaining the crop yields with smaller applications of potash. To test this possibility, forty of the fifty plots which had received no fertilizer applications for the first six crops were selected for the determination of the effects on the crop yields of applications of potash below the minimum used up to that time. The treatments used in these

plots, and the results obtained in the two crops in which said treatments were tried, are presented in table 4.

Table 5 shows the results of the statistical analysis of the yield data of table 4. In table 5 it may be seen that the differences between the mean yields of the different treatments are not significant.

It should be noted that this lack of response to the potash applications was observed in plots which had received no potash applications for the previous seven crops. The conclusion that the potash applications did not increase the crop yields at the experimental site, under the conditions

TABLE 3

Significance of differences between the mean yields obtained with different amounts of application of each fertilizer substance

FERTILIZER SUBSTANCE	TREATMENT COMPARISON	DIFFERENCE BETWEEN MEAN YIELDS	REMARK AS TO SIGNIFICANCE OF DIFFERENCE
Nitrogen	B–A	0.343	Not significant
	C–B	0.538	" "
	C–A	0.881	Significant at 5% point
Phosphoric acid	A–D	0.346	Not significant
	E–A	0.140	" "
	F–E	0.394	" "
	E–D	0.486	" "
	F–A	0.534	" "
	F–D	0.880	Significant at 5% point
Potash	I–D	0.478	Not significant
	J–I	−0.141	" "
	J–D	0.337	" "
Potash	G–A	0.035	Not significant
	H–G	0.147	" "
	H–A	0.182	" "

and for the duration of the experiment, appears to be warranted by the above results. This conclusion is also in contrast with what was to be expected from McClelland's results (2).

EXPERIMENT WITH "CATALINA CLAY" AT MAYAGUEZ

The experiment at Lares started with 16-year-old trees. On that account, data on the fertilizer requirements of young coffee trees were still lacking. To obtain desired information, the other experiment mentioned in the *Introduction* was started at Mayaguez.

The latter experiment was established on a private farm at Km. 6.1 of

road No. 13 from Mayaguez to Las Marías, with six-year-old trees of the Puerto Rican variety of *Coffee arabica*. The soil of the experimental field was also a "Catalina Clay" and the trees were planted, as in the former experiment, at a distance of eight feet between adjacent trees. "Guaba" *Inga Inga* (L) Britton, and "guama" *Inga laurina* (SW) Wild, were used to provide the shade. The plots of this experiment consisted of 16 trees each, so that each plot was 1024 square feet, or approximately 1/42.54 of an acre, in area. Each treatment was replicated seven times. The

TABLE 4

Mean yields in hundredweights market coffee per acre of the fertilizer test performed at Lares for the two crop cycle: 1940–41

TREATMENTS				REPLICATIONS										
Letter	NH_3	P_2O_5	K_2O	1	2	3	4	5	6	7	8	9	10	Total
	units	*units*	*units*											
L	15	15	0	3.43	3.06	1.69	2.87	3.30	3.32	3.65	4.27	6.10	3.73	35.42
M	15	15	5	3.06	3.57	3.19	2.95	4.72	3.37	5.00	3.91	4.50	4.22	38.49
N	15	15	10	2.54	3.56	3.32	3.39	5.65	3.89	3.38	4.55	8.00	4.13	42.41
O	15	15	15	3.92	3.16	2.91	3.25	3.68	3.17	4.72	3.30	3.08	4.84	36.03

Note: 1 unit = 7.5 pounds of substance per acre.

TABLE 5

Analysis of the total sum of squared deviations of the data of table 4

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Blocks	9	21.8684		
Treatments	3	3.0195	1.0065	1.32
Error	27	20.5625	0.7616	
Total	39	45.4504		

The differences between the treatment means are not significant.

eleven treatments tested and the results obtained in three crops harvested in this experiment are presented in table 6. The fertilizers were applied in a narrow band six inches deep, just beneath the drip of the trees and around them. In cases where the land was too steep, the band was made in a half-moon shape on the upper side of each tree. Only one fertilizer application was made for each crop, during January, after the yearly harvest.

Table 7 shows the result of the statistical analysis of the yield data of

table 6. It shows that there were significant differences between the mean yields of the treatments.

Table 8 presents the results obtained in the evaluation of the statistical significance of the yield differences that may be attributed to differences in the rates of application of the different fertilizer substances. In it, one

TABLE 6

Mean yields in hundredweights of market coffee per acre of the fertilizer test performed at Mayagüez for the three crop cycle: 1942–44

TREATMENTS				REPLICATIONS								MEAN
Letter	NH_3	P_2O_5	K_2O	1	2	3	4	5	6	7	Total	
	pounds per acre											
A	100	0	0	2.38	2.42	2.67	1.59	1.41	1.03	1.39	12.89	1.841
B	100	100	0	1.75	2.20	3.42	1.91	1.69	2.97	2.92	16.86	2.409
C	100	0	100	2.49	2.98	1.72	0.61	1.88	2.15	2.79	14.62	2.089
D	0	100	100	3.22	2.65	3.59	2.82	1.68	1.56	1.23	16.75	2.393
E	0	0	100	3.09	1.96	1.34	2.56	1.17	2.60	1.98	14.70	2.100
F	0	100	0	5.13	1.79	2.36	2.50	1.65	2.40	2.55	18.38	2.626
G	100	100	100	2.57	2.90	3.11	2.47	1.08	2.49	1.94	16.56	2.366
H	200	100	100	2.57	2.39	2.98	1.72	0.95	1.89	2.84	15.34	2.191
I	100	200	100	5.78	2.52	3.22	3.07	2.38	4.96	1.31	23.24	3.320
J	100	100	200	2.98	3.64	1.09	3.15	2.32	4.46	3.11	20.75	2.964
W	0	0	0	1.71	2.88	1.89	0.47	1.08	1.72	1.42	11.17	1.596

TABLE 7

Analysis of the total sum of squared deviations of the data of table 6

SOURCE OF THE DEVIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE ESTIMATE	F
Treatments	10	16.6823	1.6682	1.99*
Error	66	55.1900	0.8362	
Total	76	71.8723		

There are significant differences between the treatment means.
Values to be exceeded for significance between two 7-plot totals:
At the 5% point..... 0.976 hundredweights market coffee per acre
At the 1% point..... 1.297 hundredweights market coffee per acre

may see that the only fertilizer substance that has affected the yields in a significant way has been phosphoric acid. This caused significant increases when applied at the rate of 100 pounds P_2O_5 per acre, in the absence of applications of nitrogen and potash, and also when applied at the rate of 200 pounds P_2O_5 per acre, in the presence of applications of 100 pounds

each per acre NH_3 and K_2O. In all the other cases, increases in the amounts of phosphoric acid applied were associated with increases in the crop yields, though in only one of the other four cases did this increase approach significance. In none of the cases were the yields affected significantly by increases in the amounts of nitrogen and potash applied. For increases in crop yield in this field during the period covered by this test, therefore, the phosphoric acid applications proved to be necessary,

TABLE 8

Significance of differences between the total yields obtained with different amounts of application of each fertilizer substance

FERTILIZER SUBSTANCE	TREATMENT COMPARISON	DIFFERENCE BETWEEN YIELDS	REMARK AS TO SIGNIFICANCE OF DIFFERENCE
Nitrogen	A–W	0.245	Not significant
	B–F	−0.217	" "
	C–E	−0.011	" "
	G–D	−0.027	" "
	H–G	−0.175	" "
	H–D	−0.202	" "
Phosphoric acid	F–W	1.030	Significant at 5% point
	B–A	0.568	Not significant
	D–E	0.293	" "
	G–C	0.277	" "
	I–G	0.954	" "
	I–C	1.231	Significant at 5% point
Potash	E–W	0.504	Not significant
	C–A	0.248	" "
	D–F	0.233	" "
	G–B	−0.043	" "
	J–G	0.598	" "
	J–B	0.555	" "

whereas the nitrogen and potash did not exert significant effects on the crop yields.

CONCLUSIONS

Two fertilizer experiments at Lares and Mayaguez were conducted on "Catalina Clay" with the Puerto Rican variety of *Coffee arabica*.

The experiment at Lares indicated that, for maximum coffee yields, nitrogen and phosphoric acid applications were required. The experiment at Mayaguez indicated that, for maximum coffee yields, phosphoric acid applications were necessary. The experiment at Mayaguez lasted

for only three crops, however, and it should be pointed out that though the beneficial effect of the nitrogen applications on the crop yields at Lares were not statistically significant with the results of the first six crops, they were with the results of the whole nine-crop cycle. Had the experiment at Mayaguez lasted long enough, the nitrogen applications might have proved to be essential for maximum crop yields. It should be remarked that the shade trees are leguminous and, therefore, they may contribute to supply at least a portion of the crop's nitrogen requirements.

Since, however, these experiments represent but two localities, further work should be done to determine the fertilizer requirements in other sections, at other altitudes, and in some of the other important soil types as regards coffee production.

SUMMARY

The results obtained in two coffee fertilizer tests performed with the Puerto Rican variety of *Coffea arabica* on "Catalina Clay" are presented, statistically analyzed, and discussed.

Nitrogen and phosphoric acid applications seem to be of greater importance as regards market-coffee production of the above variety in the soil type used, than are the applications of potash, which had no significant effects on the yields.

These results are in sharp contrast with the results obtained by McClelland, who found potash applications to be essential and phosphoric acid applications to be not essential for maximum coffee production in Puerto Rico. It should be noted that McClelland's experiments were carried out on other soil types, which were probably not in condition to provide the coffee trees with their potash requirements.

RESUMEN

Los resultados obtenidos en dos experimentos de abono realizados con la variedad Puerto Rico de *Coffea arabica* en el suelo Catalina arcilloso han sido presentados, analizados estadísticamente y discutidos.

Las aplicaciones de nitrógeno y ácido fosfórico parecen ser más importantes en cuanto se refiere a la producción de café comercial de la variedad Puerto Rico en el suelo Catalina arcilloso que las aplicaciones de potasa, las cuales no demostraron tener efectos significativos en dichos rendimientos.

Estos resultados difieren radicalmente de los resultados obtenidos por McClelland, quien encontró que las aplicaciones de potasa eran esenciales, y que las de ácido fosfórico no eran necesarias para la producción máxima de café en Puerto Rico. Debe llamarse la atención al hecho de que McClelland realizó sus experimentos en otros suelos, los cuales probablemente no

se hallaban en condiciones de proveer al cafeto la potasa requerida por el mismo.

ACKNOWLEDGMENTS

The experiment at Lares was initiated by Mr. V. Medina, Coffee Specialist, during the year 1932. Mr. J. Guiscafré Arrillaga and L. A. Gómez conducted this experiment during subsequent years. The experiment at Mayaguez was designed and initiated by Mr. J. Guiscafré Arrillaga and continued in later years by Messrs. L. A. Gómez, E. Hernández Medina and J. Lería Esmoris. Dr. B. G. Capó cooperated in the interpretation of the results at various stages of the work, and in the preparation of this manuscript.

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TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

Part I. Effect of Lime on the Mineral Composition of the Soil, of the Grass, and on the Crop Yield

J. A. BONNET and ALFONSO R. RIERA

The land in pasture, fallow, and idle in Puerto Rico, is estimated by the 1940 Census of Agriculture to be around 776,103 "cuerdas," of which the amount of acid land dispersed in the humid area may be estimated to be about 69.3 per cent or 537,696 "cuerdas." (1 cuerda = 0.971 acre)

How the application of lime to these soils would influence the mineral composition of the soil and of the grass is not known. According to Beeson (1), fundamental studies are lacking of what changes take place in the soil when the fertilizers are supplied, and on what effect these changes will have on the plant.

This paper reports the effect that a calcium application to an acid soil has on the composition of calcium, phosphorus, magnesium, manganese, and iron of the soil, of the grass grown in this soil, and on the yield of this crop.

EXPERIMENTAL WORK

Eighteen plots, each with an area of four-tenths of an acre, were selected in a field of "Fajardo clay" at the Experiment Station Farm at Río Piedras. "Fajardo clay" is an acid red soil of the humid region, derived from old, high alluvial material and from outwash fans of adjacent shale hills. The relief is level or gently sloping.

Limestone was added on June 25, 1943 to half of the randomized plots at the rate found by the lime-requirement test reported by Riera (4). The amount of limestone applied varied from 8 to 10 tons per acre. The field was planted in the middle of July 1943 with a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, the former known as "Malojillo" and the latter as "Malojilla". Para and Carib grasses comprise the most valuable pasture and soilage grasses in the lowlands of the northeastern part of Puerto Rico.

Five consecutive crops were harvested on the dates reported in table 1. These dates varied for each crop because the grasses were cut daily, in strips, to supply to the stable herd. The grass from each strip was weighed in the field. The third and fifth crops were fertilized with ammonium sulphate at the rate of 500 pounds per acre. From January 29 to September 15, 1945, grass from the third to fifth crops inclusive, was supplied

daily to fifteen female goats used in a supplementary experiment to find the effect of the chemical composition of this grass on their health.

METHODS OF ANALYSES

SOILS

Three composite samples of the soil were taken from each plot; the first in June 1943 previous to the lime application; the second and the third, in September 1944 and May 1945, fifteen and twenty-three months, respectively, after the lime application. Each soil sample was analyzed for pH and for exchangeable calcium, magnesium, manganese, and for available iron and phosphorus.

Exchangeable Calcium, Magnesium, Manganese. Exchangeable calcium, magnesium, and manganese, were run by Peech's (3) method as follows: Weigh 10 grams of air-dried soil and leach into a 400 ml. beaker with about 225 ml. of normal neutral ammonium acetate solution. Dry leachate carefully in a hot plate and destroy organic matter and ammonium salts, adding 5 ml. of fuming nitric acid and 1 ml. of concentrated sulphuric acid and warming until the reaction has subsided and the brown fumes are no longer given off. Cool and rinse. Evaporate to dryness at low heat and continue heating for about 10 minutes to dehydrate the salts. Place the beaker in an electric muffle at 150°–200°C. and heat to 380°C. and hold at this temperature for 10–15 minutes. Treat residue with 3 ml. of 1:1 hydrochloric acid to dissolve the oxides of manganese and iron. Evaporate to dryness on steam bath and continue heating for fifteen minutes to dehydrate silica. Dissolve the salt residue with 10 ml. of 0.1 normal nitric acid. The solution should be colorless and clear, except for a trace of silica, which is either allowed to settle out in the beaker or centrifuge if necessary in a 15 ml. centrifuge tube. The solution from the beaker is decanted into a 15 ml. test-tube. This is solution A.

Transfer 2 ml. aliquot of solution A, equivalent to 2 grams of soil, to a 15 ml. centrifuge tube for the determination of calcium and magnesium. Add 0.2 ml. of ferric chloride solution (1 ml. = 1 milligram Fe), 3 ml. distilled water, and 2 ml. of 10 per cent sodium acetate solution. Mix and add 1 ml. of 0.1 normal sodium hydroxide, and mix again. Place the centrifuge tube in a water bath kept at 95°C. Add 1 ml. of a saturated solution of bromine, and maintain water bath temperature for at least one hour to flocculate the manganese dioxide, and to expel the excess of bromine. Add 2 ml. of 25 per cent ammonium chloride solution and digest for about 15 minutes. Add a drop of methyl red; and if the color of the indicator persists, indicating complete expulsion of bromine, remove the

tube from the water bath, cool, add 0.6 normal ammonium hydroxide from a burette until the color of the solution changes from a slightly red to a deep yellow; add 2 drops in excess. In general, it usually requires 0.5 ml. of 0.6 normal ammonium hydroxide. Make up to a volume of 13 ml. with water and add 5 drops of water in excess to allow for evaporation. Mix with a stirring rod and digest in water bath at 80°C. for 5 minutes to flocculate the precipitate. Centrifuge *while hot*, for 10 minutes. Designate as solution B.

Calcium. Pipette 10 ml. of solution B. equivalent to 1.5385 grams of soil, without disturbing the manganese-iron-aluminum precipitate, into a 15 ml. centrifuge tube. This is done best by holding the tube in front of a mirror. Add 0.5 ml. of 0.5 normal hydrochloric acid and 0.9 ml. of water and place in a water bath at 70°C. Mix by spinning the tube, add 2 ml. of 3 per cent ammonium oxalate. Mix thoroughly again and digest for 30 minutes at 70°C. Remove the tube from the bath and let stand for 30 minutes. The volume of the solution at this point is 13.4 ml. The excess of 0.4 ml. evaporates and the final volume of the solution is 13 ml. Decant the clear supernatant liquid into a dry test tube and keep the test-tube inverted at an angle of 45 degrees for a few minutes. Save the liquid for the magnesium determination, (Solution C). The precipitate of calcium oxalate remains in the test-tube. Add to the precipitate, 5 ml. of 2 normal ammonium hydroxide solutions saturated with calcium oxalate, break up the precipitate with a stirring rod, wash the rod, and centrifuge for 15 minutes at 1700 r.p.m. Decant the solution, drain the tube, and discard the clear liquid. Wash again, and centrifuge, if necessary. Dissolve the precipitate with 5 ml. of ten per cent sulfuric acid solution. Heat to 70°C. in a water bath and titrate with a standard 0.025 normal potassium permanganate solution.

The amount of calcium in soil is calculated as follows:

$$\text{p.p.m. Ca in soil} = (\text{ml. } KMnO_4 \times 0.025 \times 0.02004 \times 1{,}000{,}000) \div 1.5385$$
$$= 326 \times \text{ml. } KMnO_4$$

Magnesium. Take 10 ml. of solution C, equivalent to 1.1835 grams of soil, into a 15 ml. centrifuge tube. Place the tube in a bath at 70°C., add 0.8 ml. of 2 per cent alcoholic solution of 8-hydroxyquinoline, mix immediately by stirring, and then add 0.4 ml. of concentrated ammonium hydroxide from a buret. Stir vigorously for 1 minute, or longer if the amount of magnesium is extremely small, until full turbidity develops. Wash the stirring rod with a few drops of water and replace the centrifuge tube in a water bath at 70°C. for 10 minutes to flocculate the precipitate. If a number of magnesium determinations are to be carried out simultaneously,

set the centrifuge tubes aside after precipitation, until the magnesium in the last tube has been precipitated, then replace the tubes in a bath at 70°C. for 10 minutes. After digestion for 10 minutes, cool by immersing the centrifuge tubes in a bath at about 25°C., and allow to stand for 45 minutes to assure complete precipitation of magnesium; then add 0.5 ml. of 95 per cent ethyl alcohol slowly down the sides of the centrifuge tube, rotating the tube at the same time in order to wash down the precipitate and to form a layer of alcohol on the surface of the solution. Centrifuge for 15 minutes at 1700 r.p.m. and by using gentle suction draw off 2 to 3 ml. of the clear liquid to remove the layer of alcohol. Decant carefully and discard the solution; wipe the mouth of the tube with filter paper, add 5 ml. of ammoniacal ammonium acetate (8 ml. concentrated ammonium hydroxide in 300 ml. of 0.7 normal ammonium acetate), wash solution down the sides of the tube, break up the precipitate with a stirring rod, and wash the rod into the tube; add 0.5 ml. of alcohol down the sides of the tube to prevent creeping of the precipitate, and centrifuge for 15 minutes at 1700 r.p.m. Draw off the layer of alcohol, decant, and repeat the washing once more as directed above. Dissolve the precipitate in 4 ml. of 0.5 N hydrochloric acid, dilute to 13 ml. with water, stopper, and mix. Transfer a 1 ml. aliquot, equivalent to 0.0910 gram of soil to a 50-ml. volumetric flask, and add about 35 ml. of water, 5 ml. of 20 per cent sodium carbonate, and 3 ml. of phenol reagent, mixing the contents after each addition. Place the flask in boiling water for 1 minute, remove from the bath, and cool after 15 minutes. Make to volume, mix, and read in the spectrophotometer. The phenol reagent was prepared as follows: To 750 ml. of water in a 2-liter flask add 100 grams of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), 20 grams of phosphomolybdic acid ($20\ MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$), and 50 ml. of 85 per cent phosphoric acid. Boil for 2 hours, cool, and dilute to 1 liter with distilled water.

The transmittance-concentration curve (figure 1) for magnesium was developed as follows: Dissolve 0.15 gram of magnesium sulphate ($MgSO_4 \cdot 7H_2O$) in 100 ml. of 10 per cent ammonium chloride solution, heat to 60–70°C., add 10 ml. of the 8-hydroxyquinoline reagent, and make the solution alkaline with 4 ml. of concentrated ammonium hydroxide. Digest for 10 minutes, collect the precipitate on a fritted glass crucible, wash with hot dilute ammonium hydroxide, and dry at 140°C. Dissolve 0.0643 gram of the dried precipitate in 20 ml. of 0.5 normal hydrochloric acid and dilute to 500 ml. One milliliter contains 0.01 milligram of magnesium. Take 50 ml. of this standard solution and dilute to 100 ml. One milliliter of this second standard contains 0.005 milligrams of magnesium. The following transmittances were obtained, in a Coleman spectropho-

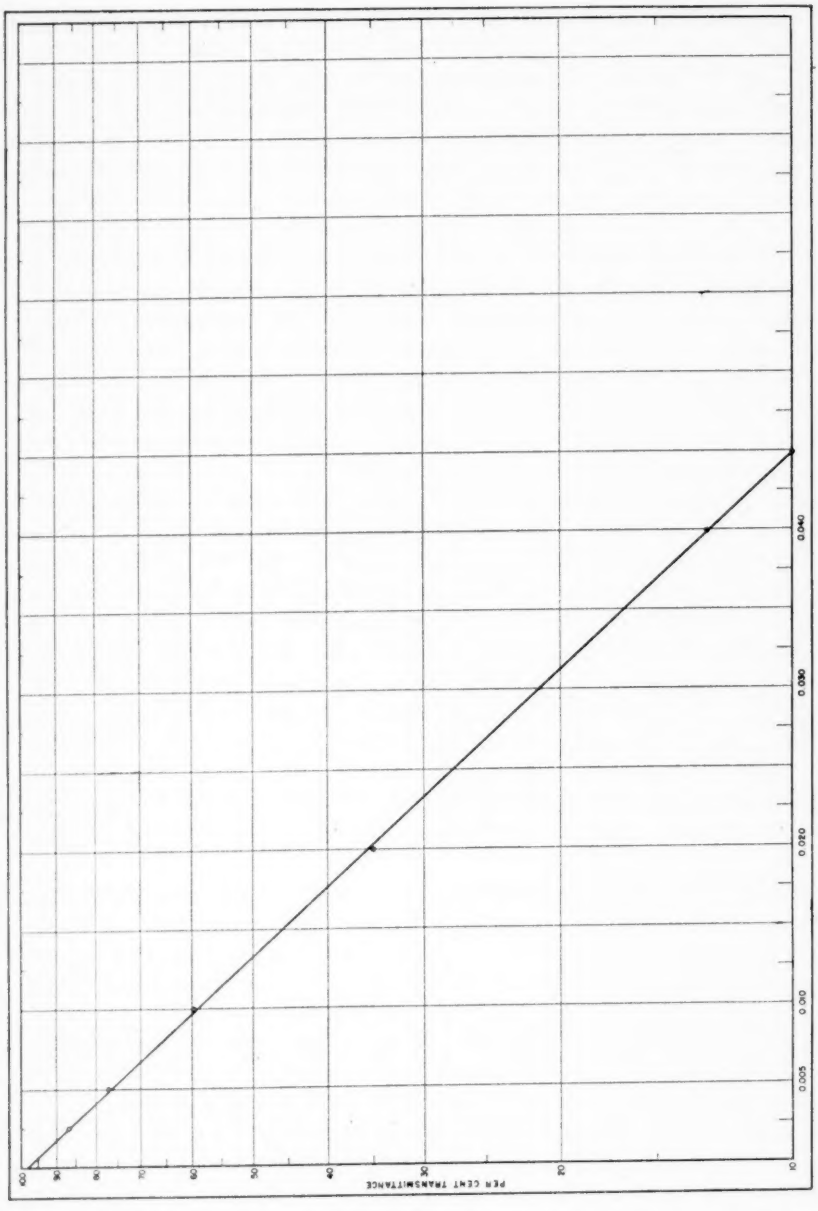

FIG. 1 Available magnesium in Soils and total magnesium in plants. Abscissa represents milligrams of magnesium as Mg.

tometer, model 11, using a PC-4 filter and a wave length of 650 mμ (figure 2), and a reagent blank as reference solution:

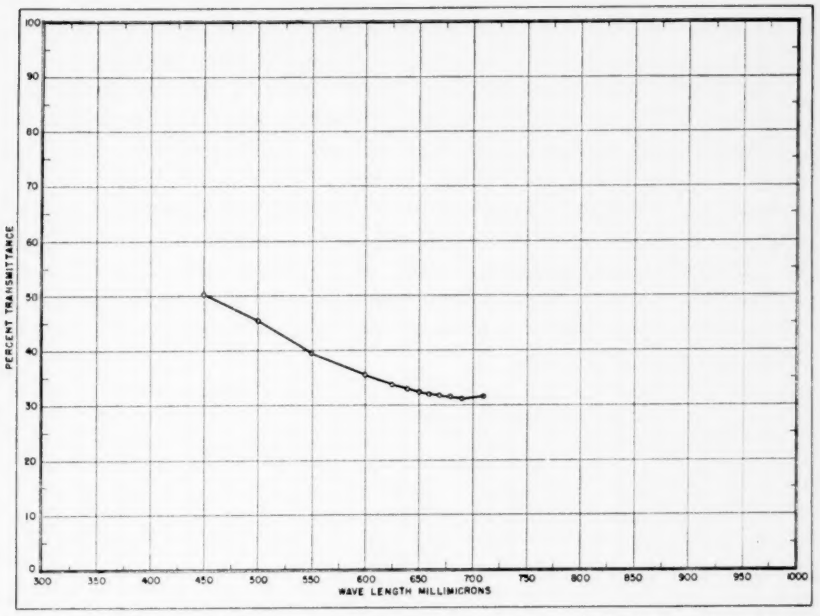

FIG. 2 Spectral-Transmittance curve for magnesium, as per method for soils and plants.

STANDARD MAGNESIUM SOLUTION		TRANSMITTANCE
ml.	*mg. Mg*	%
0.5	0.0025	87.0
1	0.005	77.2
2	0.010	59.8
4	0.020	35.1
8	0.040	12.9
10	0.050	8.9

The color was developed as explained in the procedure.

The amount of magnesium in soil is calculated as follows:

$$\text{p.p.m. Mg in soil} = \frac{\text{milligrams Mg in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0910}$$

$$= \text{mgm. Mg} \times 10{,}989$$

Manganese. Manganese was determined by the simplified periodate method described by Peech (3). Transfer 2 ml. of solution, equivalent to 2 grams of soil, to a test-tube graduated at 11 ml. Add 1 ml. of 85 per cent

phosphoric acid, dilute to 11 ml. with water, and add 0.3 ml. to allow for evaporation, and mix with a stirring rod. Place in a water bath at 95°C., add about 50 milligrams of sodium periodate, mix thoroughly agair, and leave in the bath for 1 hour to assure full color development. Cool, make to volume if necessary; mix and read in the spectrophotometer.

The transmittance-concentration curve (figure 3) for manganese was developed as follows: To 22.8 ml. of 0.1 normal potassium permanganate solution in a 250 ml. Erlenmeyer flask, add about 50 ml. of water and a few drops of concentrated sulfuric acid. Heat to boiling and reduce the permanganate by the addition of sodium sulfite until the solution is colorless. Boil off the excess of sulfur dioxide and dilute to one liter. One milliliter of this solution is equivalent to 0.025 milligrams of Mn. The following transmittances were obtained in a Coleman spectrophotometer, model 11, using a PC-4 filter and a wave length of 525 mμ (figure 4) and a reagent blank as reference solution:

STANDARD MANGANESE SOLUTION		TRANSMITTANCE
ml.	*mg. Mn*	%
1	0.025	79.8
2	0.050	63.6
3	0.075	51.0
5	0.125	33.5
8	0.200	19.1
10	0.250	13.9

The color was developed as explained in the procedure.

The amount of manganese in soil is calculated as follows:

$$\text{p.p.m. Mn in soil} = \frac{\text{milligrams Mn in curve} \times 1{,}000{,}000}{1{,}000 \times 2}$$

$$= 500 \times \text{mg. Mn in curve}$$

Available Phosphorus and Iron. Available phosphorus and iron in the soil were extracted with Morgan's Universal extracting solution, normal sodium acetate buffered at pH 4.8 with acetic acid as follows: 12.5 grams of air-dried soil and 25 ml. of extracting solution were placed in a test-tube, 6″ long and 1″ in diameter. The tube was stoppered and shaken horizontally for 2 minutes, in a reciprocating shaker (Amer. Instrument Co. cat. #7-155) at a speed of about 120 shaking cycles per minute. The extract was filtered in a Whatman filter paper No. 1.

Available Phosphorus—Phosphorus was precipitated as ammonium phosphomolybdate, reduced to the blue color with aminonaphtholsulfonic acid and determined colorimetrically as per Wolf's (5) procedure as follows: Take an aliquot of 5 ml. of soil extract, equivalent to 2.5 grams of soil,

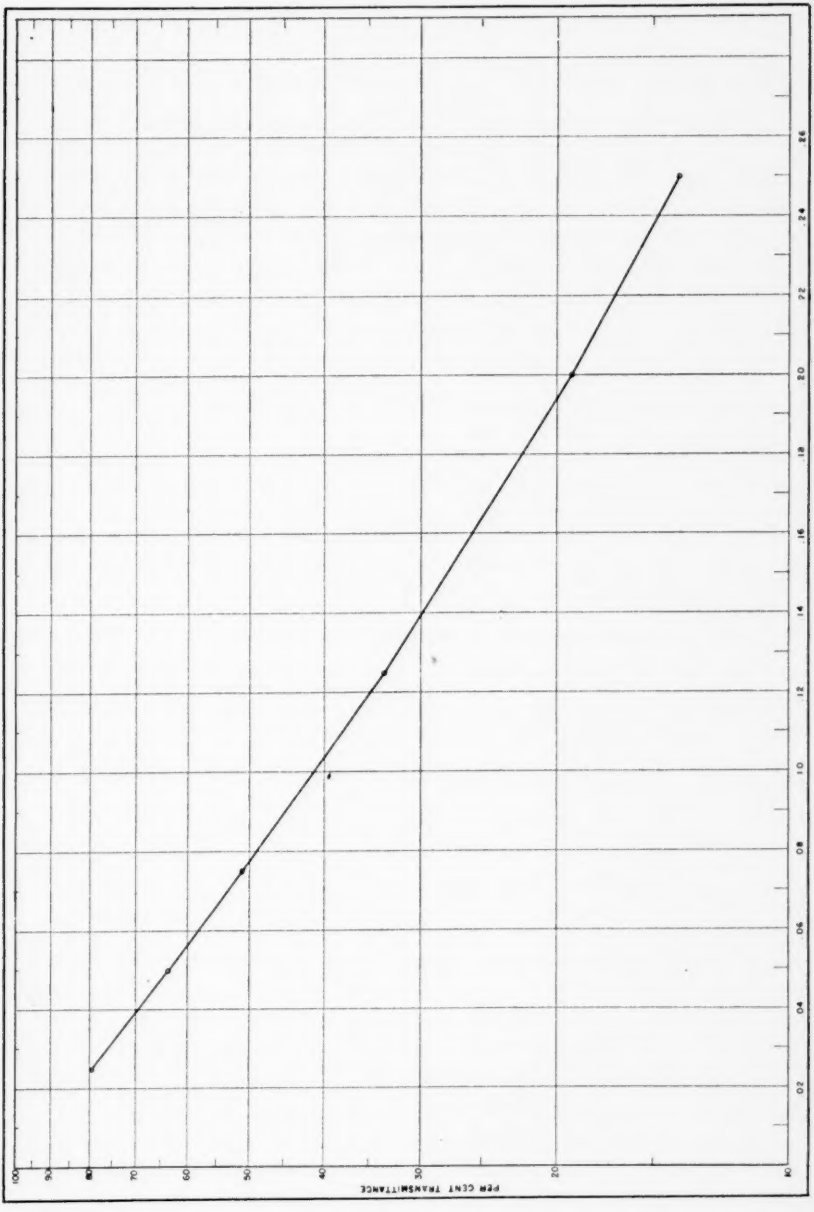

FIG. 3 Available manganese in soils and total manganese in plants. Abscissa represents milligrams of manganese as Mn.

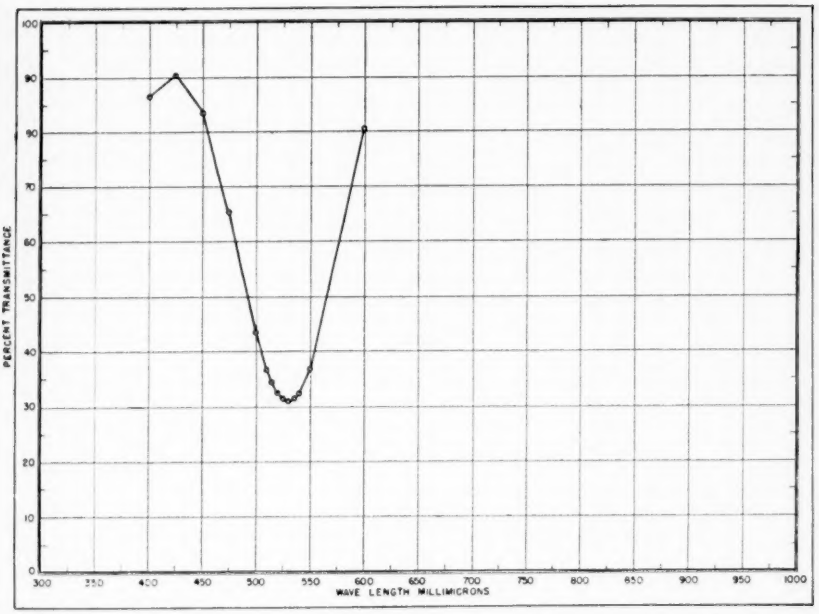

FIG. 4 Spectral-Transmittance curve for manganese as per method for soils and plants.

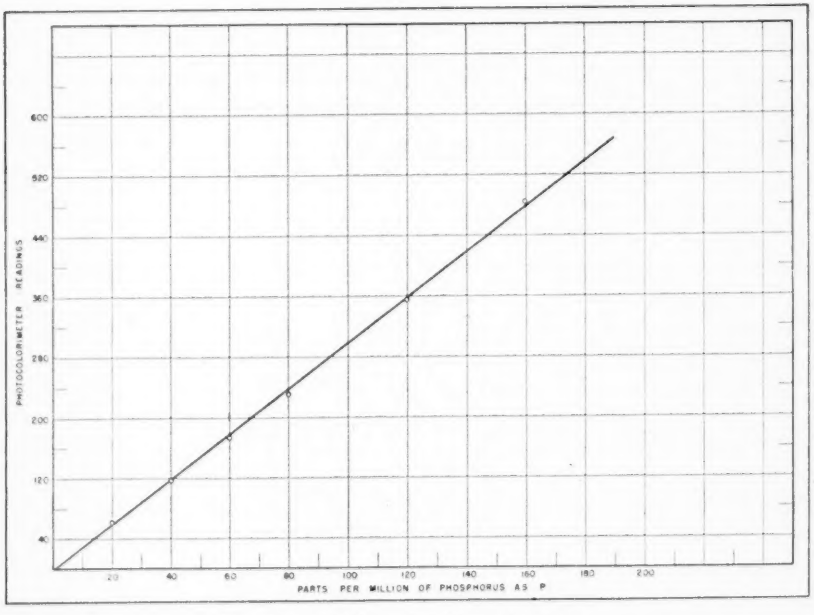

FIG. 5 Available phosphorus in soils.

dilute to 20 ml. with extracting solution, add 4 ml. of ammonium molybdate solution (2.5 per cent in 6 normal sulfuric acid, and 2 ml. of aminonaphthol-sulfonic acid solution (15 grams of anhydrous sodium bisulfite, are dissolved in 100 ml. of water, and 0.5 gram of pure, dry 1-amino-2-naphthol-4-sulfonic acid, and 1.5 grams of anhydrous sodium sulfite, are added; shake, make up to 500 ml. and store in a brown bottle).

The concentration curve for phosphorus (figure 5) was obtained in a Klett-Summerson photoelectric colorimeter No. 2141 with red filter 66 covering wave lengths from 640 to 700 mμ and instrument set at zero with reagent blank. The procedure was as follows: Weigh 0.1006 grams of sodium monobasic phosphate ($NaH_2PO_4 \cdot 2H_2O$) and dissolve in one liter of water. One milliliter of this solution is equivalent to 20 parts P per million. The following readings were obtained in the photoelectric colorimeter:

STANDARD PHOSPHORUS SOLUTION			PHOTOCOLORIMETER READING
ml.	*mg. P*	*p.p.m. P*	
1	0.02	20	60.5
2	0.04	40	115.4
3	0.06	60	174.4
4	0.08	80	229.9
6	0.12	120	353.5
8	0.16	160	483.0

The slope of this curve was found not to be constant. To check the slope, three phosphorus standards should be run with the unknown.

The amount of phosphorus in soil is calculated as follows:

$$\text{p.p.m. available P in soil} = \text{p.p.m. P in curve} \times \frac{1}{2.5}$$

$$= \text{p.p.m. P in curve} \times 0.4$$

Available Iron—An aliquot of 1 ml. of the soil extract equivalent to 0.5 gram of soil, was poured in a test-tube graduated at 10 ml. The color was developed as per method of Saywell and Cunningham, described by Parks et al (2), as follows: Add 1 ml. of 10 per cent hydroxylamine hydrochloride solution and 0.5 ml. of ortho-phenanthroline (1.5 per cent in 95 per cent ethanol), make to volume, mix and read in the photoelectric colorimeter. As the original extract was buffered to pH 4.8 there was no need of adjusting the pH with ammonium hydroxide as mentioned by Parks.

The concentration curve (figure 6) for iron was developed as follows: Weigh one-gram of c.p. iron wire in a liter volumetric flask and dissolve in about 150 ml. of 1:6 sulfuric acid; add 5 ml. of concentrated nitric acid as oxidizing agent; boil to expel SO_3 fumes, and complete volume to one liter.

One milliliter of this solution is equivalent to one milligram Fe. Ten milliliters of this solution were diluted to one liter; one milliliter of this solution contains 0.01 milligrams Fe. The following readings were obtained in a Klett-Summerson photoelectric colorimeter, No. 2141, with blue filter 42

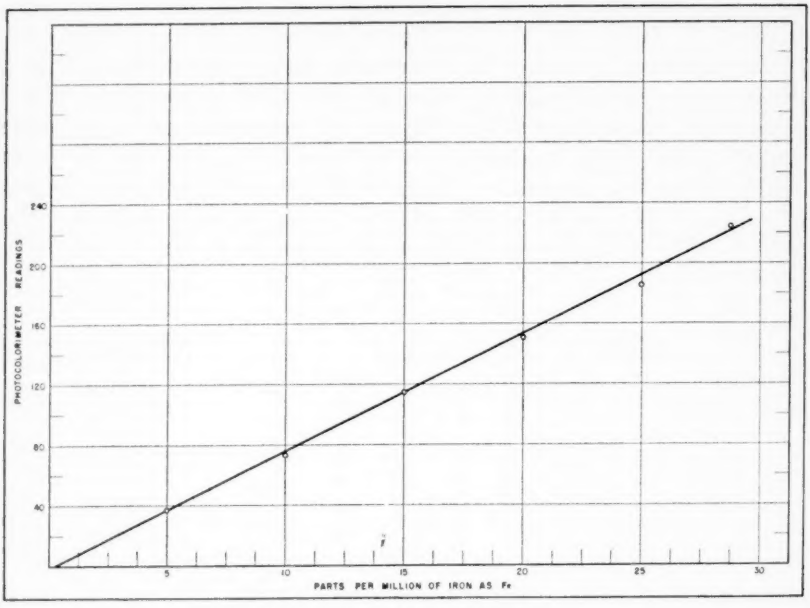

FIG. 6 Available iron in soils.

covering wave lengths from 400–465 mμ, and the colorimeter set at zero with reagent blank:

STANDARD IRON SOLUTION			PHOTOCOLORIMETER READING	SLOPE FACTOR
ml.	*mg. Fe*	*p.p.m. Fe*		
0.5	0.005	5	37	.1351
1.0	0.010	10	73	.1370
1.5	0.015	15	115	.1304
2.0	0.020	20	151	.1325
2.5	0.025	25	184	.1351
3.0	0.030	30	224	.1339

The color was developed as explained in the procedure. The slope of the curve was found to be constant; its average value is 0.1340.

p.p.m. available Fe in soil = p.p.m. Fe in curve × 2
= photocolorimeter reading × 0.1340 × 2
= 0.268 × photocolorimeter reading

GRASS

A composite sample (about ten pounds) of the standing grass was taken from each plot. The samples were dried to constant weight in a hot air oven at 150°F. Each sample was ground in a Wiley mill and sifted through a 1 mm. sieve. The ground samples were left overnight at room temperature to absorb atmospheric moisture. The dates of grass samplings are reported in table 1.

A 7.50 gram sample of dry grass was weighed in a 600 ml. pyrex beaker for each determination and the procedure of Parks et al (2), omitting the dithizone extraction, was followed as explained below.

Destruction of Organic Matter and Removal of Silica. Destroy the organic matter with nitric and perchloric acids; add first 12.5 ml. of concentrated nitric acid, place a cover glass on top of the beaker, and heat in a steam plate or hot plate at low temperature, in the hood; add again 12.5 ml. of nitric

TABLE 1

Dates of soil and grass samplings, of fertilizer application, of harvesting, and age of grass at harvest time

CROP	DATES OF SOIL SAMPLING	DATES OF GRASS SAMPLING	DATES OF AMMONIUM SULPHATE APPLICATION	HARVESTING DATES	AGE OF CROP HARVESTED	
					At start	At end
					mo.	*mo.*
First	6/43			1/13–27/44	5.5	6.0
Second	9/44	8/28/44		8/28–11/14/44	7.0	9.5
Third		11/16/44	10/10/44	11/15/44–3/29/45	2.5	4.5
Fourth	5/45	4/10/45		3/30–7/29/45	4.5	4.0
Fifth		7/16/45	4/25/45* 6/5/45*	7/30–10/25/45	4.0	3.0

* Only one application; dates refer to application for half of each plot.

acid and evaporate to near dryness. Add to residue 25 ml. of concentrated nitric acid and 25 ml. of 60 per cent perchloric acid. Do not add the perchloric acid before the nitric acid treatment because an explosion may occur. Evaporate to near dryness. Transfer residue quantitatively into a 125 ml. platinum dish, washing four or five times with 5 ml. portions of water. Add 5 to 8 ml. of 48 per cent hydrofluoric acid, from an 8 ml. beaker coated with paraffin, to the platinum dish; heat in hot plate carefully to dryness until silicon fluoride fumes are totally driven off. While working with grass samples from the dry area of Puerto Rico, a pink color persisted in this stage. It was destroyed by adding a pinch of peroxydisulfate ($K_2S_2O_8$) salt and a few drops of concentrated nitric acid. Cool, add 10 ml. of hot 0.6 normal hydrochloric acid, and dissolve the salts by continued heating and crushing of solid material with a flat end glass rod. Transfer to a 100 ml.

volumetric flask. Repeat heating-crushing operation, until the salts go in solution. Make up to 100 ml. volume with water and label, "Solution A"; 1 milliliter of this solution is equivalent to 0.075 gram of plant tissue.

Manganese. Manganese was determined by the simplified periodate method described by Peech (3). Pipette a 10 ml. aliquot of "Solution A" equivalent to 0.75 gram of plant tissue, into a 50 ml. beaker and evaporate to dryness, in a hot plate, to remove excess of hydrochloric acid. Dissolve residue in 6 ml. of normal nitric acid and transfer to a test-tube graduated at 11 ml. and follow the procedure explained before for the soils.

The transmittance-concentration curve (figure 3) was also developed as explained for the soils.

The amount of manganese in plant is calculated as follows:

$$\text{p.p.m. Mn in plant} = \frac{\text{milligrams Mn in curve} \times 1{,}000{,}000}{1000 \times 0.75}$$

$$= 1333 \times \text{mg. Mn in curve.}$$

Iron. Pipette 1 ml. of "Solution A" equivalent to 0.075 gram of plant tissue into a test-tube graduated to 10 ml. and develop color as explained before for soils.

Transmittance was measured this time in a Coleman spectrophotometer, model 11. The transmittance-concentration curve for iron (figure 7) was determined in the same standard used for soils. The following transmittances were obtained with filter PC-4, at a wave length of 490 mμ, using a reagent blank as reference solution:

STANDARD IRON SOLUTION		TRANSMITTANCES
ml.	*mg. Fe*	%
0.5	0.005	79.2
1.0	0.010	61.3
1.5	0.015	48.1
2.0	0.020	37.4
2.5	0.025	30.0
3.0	0.030	24.0

The amount of iron in plant is calculated as follows:

$$\text{p.p.m. Fe in plant} = \frac{\text{milligrams Fe in curve} \times 1{,}000{,}000}{1{,}000 \times 0.075}$$

$$= 13{,}333 \times \text{mg. Fe in curve.}$$

Phosphorus. Pipette a 0.1 ml. of solution A, equivalent to 0.0075 gram of plant tissue into a test-tube graduated at 10 ml. using a 0.1 ml.

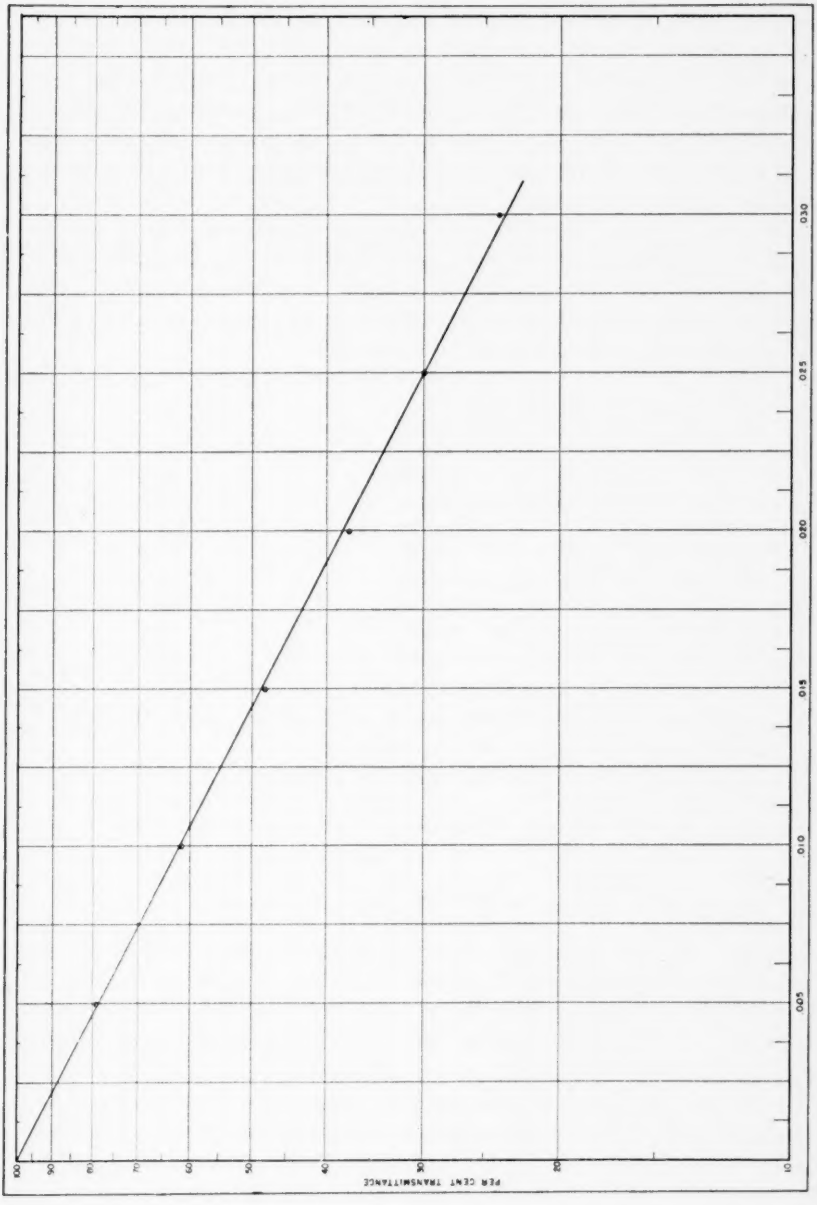

FIG. 7 Iron in plants. Abscissa represents milligrams of iron as Fe.

Mohr's pipette. Add 1 ml. of water and discharge the pipette by blowing with the tip under the water. Add 1 ml. of ammonium molybdate solution (2.5 per cent in 5 normal sulfuric acid), mix, and add 0.4 ml. of 0.25 per cent aminonaphtholsulfonic acid solution (0.125 gram of aminonaphtholsulfonic acid to 49 ml. of filtered 15 per cent sodium bisulfite, and then adding 1.25 ml. of 20 per cent sodium sulfite). Make to volume and mix. Read transmittance in spectrophotometer.

The transmittance-concentration curve for phosphorus (figure 8) was obtained in a Coleman spectrophotometer, model 11, with filter PC-4, at a wave length of 600 mμ, using distilled water as reference solution. The following transmittances were obtained in eight phosphorus standard solutions prepared as explained in the soils procedure:

STANDARD PHOSPHORUS SOLUTION		TRANSMITTANCE
ml.	*mg. P*	%
1.0	0.0050	83.1
1.5	0.0075	78.5
2.0	0.0100	72.9
3.0	0.0150	63.0
3.5	0.0175	58.6
4.0	0.0200	54.1
5.0	0.0250	46.7
5.5	0.0275	43.5

The slope of this curve was found not to be constant. It is suggested to run three standard solutions with the unknown.

The calculation of phosphorus in plant is as follows:

$$\text{p.p.m. P in Plant} = \frac{\text{milligrams P in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0075}$$

$$= 133{,}333 \times \text{mg. P in curve.}$$

Removal of Iron, Aluminum, and Phosphorus Previous to Calcium and Magnesium Determinations. Transfer a 2.0 ml. aliquot of solution A, equivalent to 0.15 gram of plant tissue, to a 15 ml. centrifuge tube graduated at 13 ml. Add 0.2 ml. of ferric chloride solution (1.22 grams of ferric chloride hexahydrate in 250 ml. of 1 to 250 hydrochloric acid), mix, add 8 ml. of buffer solution (25 grams of sodium acetate, 62.5 grams of ammonium chloride, and 0.5 gram of sodium hydroxide in 1 liter of solution), and mix again. Add 1 drop of methyl red indicator solution (0.02 per cent) and 0.6 N ammonium hydroxide until the color of the solution changes from slightly red to deep yellow, and then add 2 drops in excess. Dilute to about 13.2 ml., mix with a stirring rod, and digest in a water bath at 80°C. for 5 minutes to flocculate the precipitate. Mix thoroughly, and centrifuge

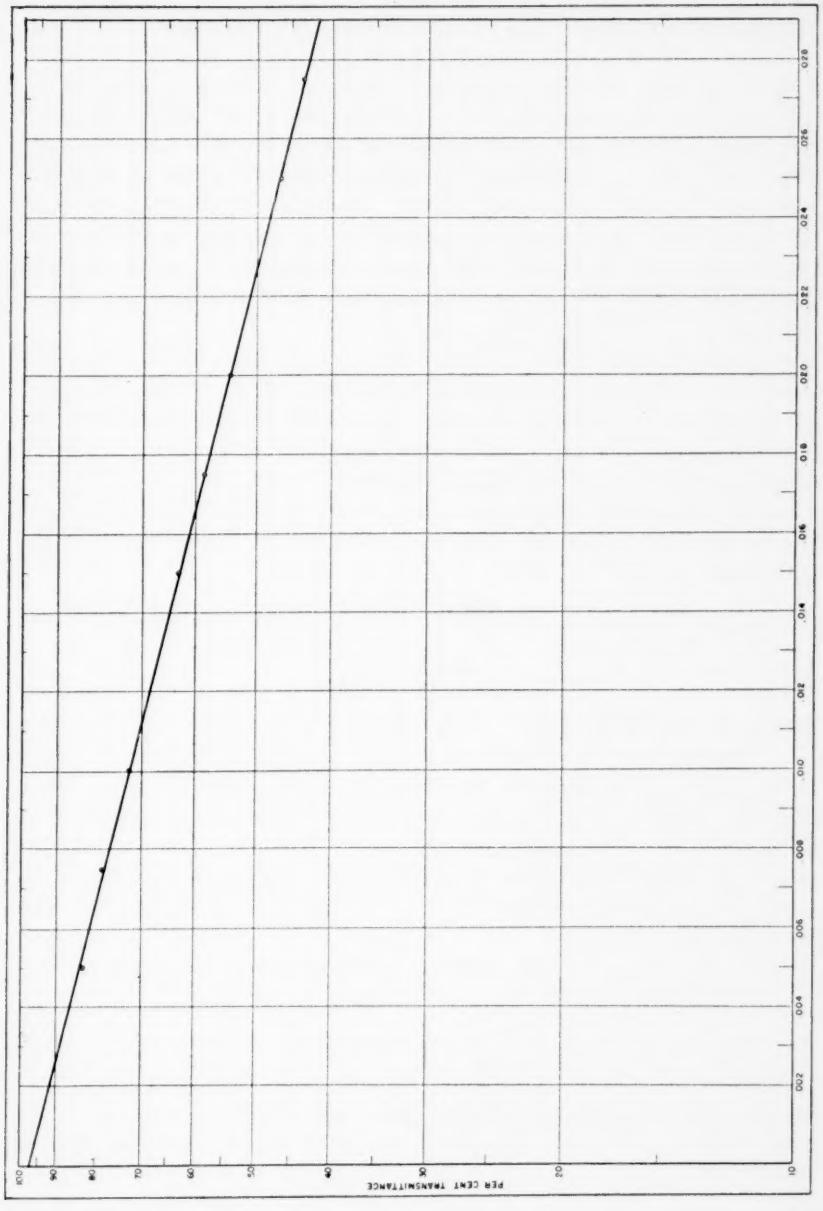

FIG. 8 Phosphorus in plants. Abscissa represents milligrams of phosphorus as P.

while hot for 10 minutes. The solution should have evaporated to 13.0 ml.

Calcium. Calcium was determined by the method of Peech (3). Transfer a 10 ml. aliquot from the above 13 ml. clear solution, equivalent to 0.1154 gram of plant tissue, into another calibrated 15 ml. centrifuge tube; add 1.4 ml. of 0.2 normal hydrochloric acid, and place in a water bath at 70°C. Mix; add 2 ml. of 3 per cent ammonium oxalate, mix thoroughly again, and digest for 30 minutes at 70°C. Remove the tube from the bath and let stand for 30 minutes. Centrifuge for 15 minutes, at about 2000 r.p.m. The volume should now be 13 ml. Decant the clear supernatant liquid gently into a 25 ml. test-tube and save for the magnesium determination.

Allow the centrifuge tube to drain for several minutes, inclined at a 45° angle, on a filter paper. Add quickly from a pipette, about 5 ml. of 2 normal ammonium hydroxide saturated with calcium oxalate, centrifuge for 15 minutes, decant carefully and discard the solution. Drain the tube and save the precipitate. One washing is sufficient unless very large quantities of calcium are present. Add about 5 ml. of 10 per cent sulfuric acid, heat to 70°C. on a water bath, and titrate with standard 0.025 normal potassium permanganate.

$$\text{p.p.m. Ca in plant} = \frac{\text{ml. } KMnO_4 \times 0.025 \times 0.02004 \times 1{,}000{,}000}{0.1154}$$

$$= 43{,}413 \times \text{ml. } KMnO_4$$

Magnesium. Magnesium was also determined by the method of Peech (3). Pipette 10 ml. of supernatant liquid, from the solution set aside for the magnesium determination, equivalent to 0.0887 gram of plant tissue, in a 15 ml. centrifuge tube graduated at 13 ml. and proceed as described in the magnesium determination reported before for soils. Take a 2 ml. aliquot from the 13 ml. solution, equivalent to 0.01365 gram of plant tissue, and develop color as mentioned previously for soils. Read transmittance in curve (figure 1).

$$\text{p.p.m. Mg in plant} = \frac{\text{milligrams Mg in Curve} \times 1{,}000{,}000}{1{,}000 \times 0.01365}$$

$$= 73{,}260 \times \text{mg. Mg in Curve}$$

Proteins, Ether Extract and Fiber. Proteins, ether extract, and fiber were determined in the first, second and third crops. Proteins were also determined in the fourth crop, previous to and after the second application of ammonium sulphate.

PRESENTATION AND DISCUSSION OF DATA OBTAINED

The mineral changes brought about in the soil, fifteen and twenty-three months after the lime application, are expressed in table 2.

The increase of available calcium and phosphorus and the decrease of available iron in the soil due to liming, was highly significant, fifteen and twenty-three months after the lime was applied to the soil. The decrease of available manganese in the soil due to liming was highly significant fifteen months after liming and significant twenty-three months after liming. The difference between the available magnesium content of the limed and unlimed soil was not significant.

TABLE 2

Parts per million of available calcium, magnesium, manganese, phosphorus, and iron in soil type Fajardo clay, unlimed and limed (dry basis)

TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
15 months after liming	Unlimed	849	180	42	13	17
	Limed	6831	172	8	61	2
23 months after liming	Unlimed	992	156	29	21	45
	Limed	5351	156	5	56	12

TABLE 3

Parts per million of calcium, magnesium, manganese, phosphorus and iron in three crops of Para-Carib grass grown in soil Type Fajardo Clay, unlimed and limed (air dry basis)

CROP NUMBER	TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Second	14 months after liming	Unlimed	2199	1509	229	2100	149
		Limed	2811	1638	137	2430	158
Third	17 months after liming	Unlimed	2008	1824	156	2749	196
		Limed	3351	2212	84	3047	160
Fourth	32 months after liming	Unlimed	2919	2166	243	2450	124
		Limed	3381	2088	181	2929	121

The mineral changes brought about in the grass after the lime application are expressed in table 3.

The increase of calcium and the decrease of manganese in the grass due to liming was highly significant for the second and third crops while the increase of calcium was significant for the fourth crop, and the decrease of manganese was not significant. The increase of phosphorus in the grass due to liming was highly significant for the second and fourth crops but was not significant for the third crop. There was no significant change in the iron

content of the grass due to liming in the three crops and in the magnesium content of the second and fourth crops. However, the increase of magnesium in the grass crop due to liming was highly significant for the third crop.

The average total yield of green grass per acre in the unlimed and limed soil for each of the first five consecutive crops, and for the five crops, is reported in table 4.

The increase in the grass yield due to liming was significant for the first and third crops. However, the difference between the respective yields of the unlimed and limed soil for the second, fourth and fifth crops, and for the total of five crops, was not significant.

TABLE 4

Yield in tons per acre of green Para-Carib grass in Fajardo clay unlimed and limed

TREATMENT	NUMBER OF CROP					TOTAL
	1 No nitrogen applied	2 No nitrogen applied	3 Nitrogen applied	4 No nitrogen applied	5 Nitrogen applied	
Unlimed	8.98	7.47	9.59	8.92	9.82	44.78
Limed	11.00	8.03	10.33	8.62	9.81	47.79

TABLE 5

Grass yields of table 4 expressed as tons per acre per month of green grass

TREATMENT	NUMBER OF CROPS AND AGE IN MONTHS				
	1 5.75 mo.	2 8.25 mo.	3 3.50 mo.	4 4.25 mo.	5 3.50 mo.
Unlimed	1.56	.91	2.74	2.10	2.81
Limed	1.91	.97	2.95	2.03	2.80

The monthly rate of growth for each of the five grass crops is reported in table 5. The age of the crop used for this calculation was the mean of that reported in table 1.

The increase in the yield of the third grass crop is due to nitrogen fertilization. It gave about two tons of green grass more per acre than the previous crop (table 4). The monthly rate of growth was about three times higher (table 5). In a period of 7.75 months, the third and fourth crops combined gave close to 5 tons of green grass per acre, while the unfertilized second crop in 8.25 months gave about one ton. In fourteen months the first two unfertilized crops gave about three tons of green grass per acre.

However, in about a period of one year the last three crops gave about eight tons of green grass per acre. The eight-ton year yield was obtained with two applications of nitrogen fertilizer, one to the third crop and another to the fifth crop, each at the rate of 500 pounds of ammonium sulphate per acre.

The increase of grass yield is not the only advantage obtained when nitrogen is applied. The content of the nitrogen in the crop is also increased if the grass is cut early (table 6).

The protein content of the Para-Carib grass mixture ranged between 3 and 4 per cent. Grass from the third crop taken 36 days after the first nitrogen application gave around 11 per cent protein or about three times that in the original grass. The ammoniacal content in the third crop was .07 and .05 per cent, respectively, for the unlimed and limed grass. The protein content of the fifth grass crop, collected 82 days after the nitrogen application, was about 5 per cent.

TABLE 6

Protein content of Para-Carib grass in five consecutive crops, before and after nitrogen fertilization (air-dried-basis)

TREATMENT	CROP 1, NO NITROGEN APPLIED	CROP 2, NO NITROGEN APPLIED	CROP 3, 36 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 4, 180 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 5, 82 DAYS AFTER SECOND NITROGEN APPLICATION
	%	%	%	%	%
Unlimed	3.6	3.8	11.7	3.3	4.8
Limed	3.8	3.7	10.8	3.6	4.5

SUMMARY

This paper reports the procedures followed for the chemical determinations of exchangeable calcium, magnesium, and manganese; and available phosphorus and iron in soils; and for the total amount in plants of each of those minerals mentioned. Spectrophotometric methods are given for magnesium and manganese in soils and plants; and for phosphorus and iron in plants including the transmittance-concentration and spectral-transmittance curves for each of these elements. Photocolorimetric methods are also given for available iron and phosphorus in soils with their corresponding curves.

This paper reports also changes of the minerals calcium, magnesium, manganese, phosphorus and iron in an acid soil, 15 and 23 months after liming. It also reports changes of these minerals in each of five crops of a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, grown in the unlimed and limed soil. The yield of green grass is also reported for each crop.

The important results are as follows:

1. Significant increases of available calcium and phosphorus and significant decreases of available manganese and iron in the soil, due to liming, and no significant difference of the available magnesium content.
2. Significant increase of calcium and no significant decrease of iron in each of three consecutive crops of the grass, due to liming. Significant decrease of manganese in the first two crops but no significant difference in the third crop. No significant difference in the magnesium content of the first and third crops but a significant difference in the middle crop.
3. The increase in the grass yield, due to liming, was significant for the first and third crops but was not significant for the second, fourth, and fifth crops, or for the total of the five consecutive crops.
4. An application of 500 pounds of ammonium sulphate per acre gave about two tons of green grass more per acre than a previous unfertilized crop. The period of growth of the fertilized grass was 3.5 months while that of the unfertilized grass was 8.25 months.
5. Grass collected early, 36 days after the nitrogen application, contained around 11 per cent of protein or about three times as much as in the unfertilized grass.

ACKNOWLEDGMENT

The writers wish to express their appreciation to Miss Gloria María Soto, former Junior Chemist of the Soils Department, for technical help on some analyses of the soils and grass samples.

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TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

Part II. Effect of Unlimed and Limed Grass on the Chemical Composition of Goats' Blood

J. A. BONNET, A. R. RIERA, L. RIVERA BRENES and R. ORLANDI

The effect of lime applications on the composition of calcium, magnesium, manganese, phosphorus and iron, in the acid red soil type "Fajardo clay", and on a mixture of "Para and Carib" grass, was discussed in Part I published by Bonnet and Riera (1).

This paper presents information on the effect of the unlimed grass, the limed grass, and the limed grass supplemented with manganese per os, on the weight of the goat; on the grass consumed; and on the hemoglobin, calcium, phosphorus, iron, hematocrits, red-blood cells, and white-blood cells of the goats' blood.

EXPERIMENTAL

The layout of the experimental field was explained in Part I. All the grass cut daily from the strips of the unlimed plots was chopped into small pieces and mixed into a sample labeled "Unlimed grass". An identical sample from the limed plots was labeled "Limed grass".

Fifteen one-year virgin female goats were selected for the experiment. They were given the parasite treatment: twelve grams of phenothiazine per os. The animals were randomized, one for each of fifteen pens (see photo), into five groups for the following three treatments: 1) goats fed with unlimed grass, 2) goats fed with limed grass, 3) goats fed with limed grass, and in addition, fed per os, with manganese sulphate.

The goat experiment covered an eleven-month period. It was started on October 19, 1944 and finished on September 15, 1945. The experiment was divided into four periods as follows:

1. Pre-feeding period (October 19–November 14, 1944)
2. Pre-gestation period (Nov. 15, 1944–January 15, 1945)
3. Gestation period (January 16–July 15, 1945)
4. Lactation period (July 16–September 15, 1945)

Eight pounds of the chopped "Unlimed grass" were fed to each of the goats in treatment No. 1. Eight pounds of the chopped "Limed grass" were fed to each of the goats in treatments No. 2 and No. 3. The feeding box (see photo) in each pen avoided the contamination of the grass with urine and excrement.

Each animal in treatment No. 3 was supplied daily in addition, per os, a solution containing 0.453 gram of manganese sulphate ($MnSO_4H_2O$) per liter, equivalent to .147 milligrams of manganese per milliliter. From November 15 to November 29, 1944, inclusive, and from February 3, 1945 to July 15, 1945, inclusive, each goat received daily, per os, 1 ml. of the manganese solution, but for the period between November 30, 1944 to February 2, 1945, inclusive, each goat received daily, per os, 1.5 ml. of the manganese solution. For the eight-month period, covering pre-gestation and gestation, each goat received 275.5 milliliters of the manganese solution equivalent to 40.5 milligrams of manganese.

The amount of residual grass left daily by each animal was also weighed. A composite sample from the "Unlimed Grass" and the "Limed Grass", was taken daily for moisture analysis. A record was kept of the amount of green and dry grass consumed by each animal.

Rain water from the two concrete wells besides greenhouse No. 5 in the Experiment Station Farm, was supplied daily to each animal. The water consumed daily, however, was very low. The mineral content of this water was as follows:

	parts per million
Calcium	4.0
Phosphorus	2.0
Iron	0.03
Magnesium	None
Manganese	None

Each goat was weighed three times in three consecutive days, around the middle and the end of each month.

To induce breeding of the goats at approximately the same time, each animal was given, per os, on January 29, 1945, 5 milligrams of diethyl stylbestrol.

METHODS OF ANALYSES

Blood samples were taken from each animal at the beginning of the "Pre-Feeding Period" on October 19, 1944; one month after the beginning of the "Pre-Gestation Period" in the middle of December 1945; and thereafter every middle of the month up to August 1945. About 10 ml. of blood were drawn from each animal by a direct puncture of the jugular vein: 2 ml. for the hematological test and 8 ml. for the chemical test. The 2 ml. blood portion was poured into a 10 ml. test tube containing a dry oxalate salt. This salt was prepared by adding 0.1 ml. of a mixed solution of 6 per cent ammonium oxalate and 4 per cent of potassium oxalate to each tube, and evaporating to dryness.

Hematological Test

A 0.1 ml. of the oxalated blood was used for the red-blood cell and white-blood cell counts. A 0.7 ml. portion of this blood was used for hematocrits and 0.1 ml. for hemoglobin.

Hemoglobin

Hemoglobin was determined in a fresh sample of cow's blood by the Van Slyke's method (2). Its content was found to be 9.04 grams hemoglobin per 100 ml. blood. A 1:25 solution was prepared by diluting 2 ml. of this cow's blood to 50 ml. with 0.1 per cent sodium carbonate solution. Transmittances of eleven dilute solutions, prepared from the 1:25 blood solution, are as follows:

SOLUTION NO.	ALIQUOT 1:25 BLOOD SOL	DILUTED WITH 0.1% SODIUM CARBONATE TO	DILUTION RATIO	HEMOGLOBIN MILLIGRAMS PER ML. BLOOD	TRANSMITTANCE
	ml.			*mg.*	%
1	6.00	18.00	1: 75.0	1.2053	15.0
2	5.00	20.00	1: 100.0	0.9040	22.5
3	5.00	23.00	1: 114.7	0.7881	26.7
4	5.00	26.67	1: 133.3	0.6782	31.3
5	5.00	32.50	1: 162.5	0.5563	38.1
6	2.50	20.00	1: 200.0	0.4520	45.1
7	2.50	26.67	1: 266.7	0.3390	54.8
8	1.25	20.00	1: 400.0	0.2260	66.8
9	1.25	26.67	1: 533.4	0.1695	73.6
10	1.25	40.00	1: 800.0	0.1130	81.0
11	1.25	80.00	1:1600.0	0.0565	90.0

The transmittance was determined in a Coleman spectrophotometer, model 11, using a PC-4 filter at a wave length of 540 $m\mu$ where maximum color absorption (figure 2) occurs. The sodium carbonate solution was used as reference. It gave a 99.1 per cent transmittance when the instrument was set at zero. The transmittance-concentration curve obtained for hemoglobin is reported in figure 1.

For the determination of hemoglobin in the unknown, 0.1 ml. of oxalated blood was diluted to 20 ml. with 0.1 per cent sodium carbonate solution. The transmittance of the colored solution was read in a Coleman spectrophotometer, model 11, using filter PC-4 at a wave length of 540 $m\mu$. The transmittance from the curve (figure 1) multiplied by 20 gives grams of hemoglobin per 100 ml. blood.

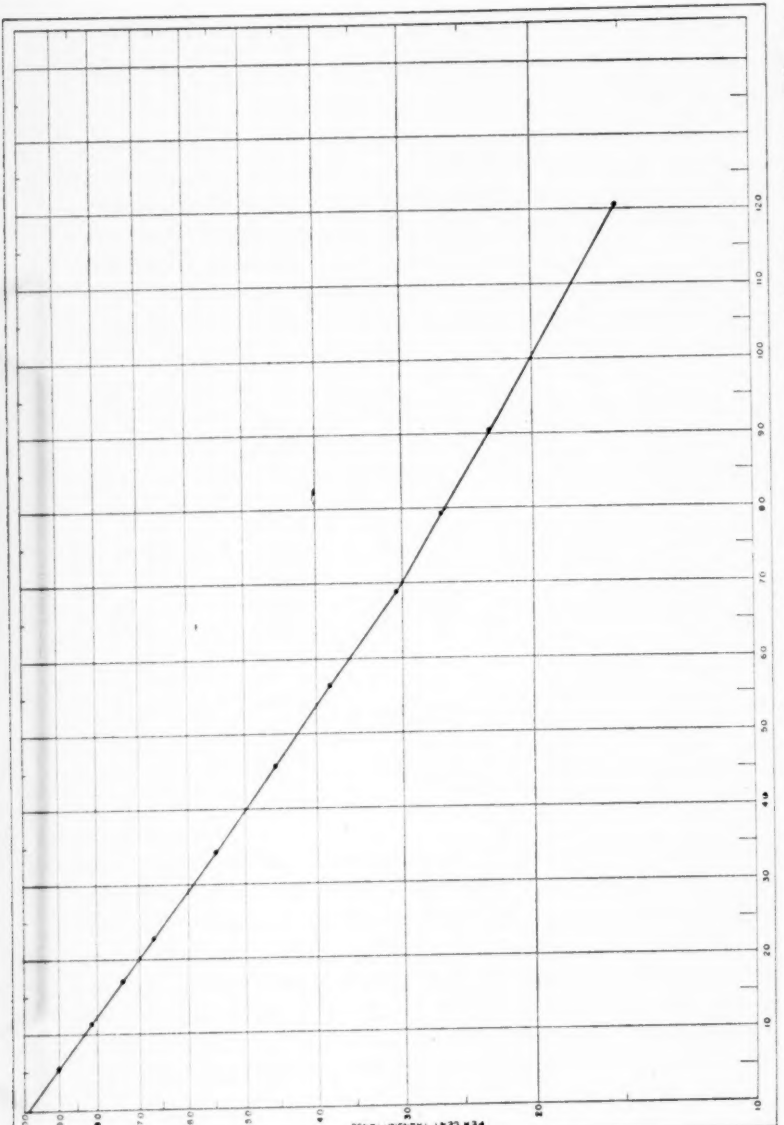

FIG. 1. Hemoglobin in blood. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 540 mu, and 0.1 per cent sodium carbonate as reference solution. Abscissa represents milligrams of hemoglobin per milliliter blood. On the basis of 0.1 ml. blood diluted to 20 ml. with 0.1% $NaCO_3$; value in curve $\times 20$ = grams hemoglobin per 100 ml. blood.

Chemical test

Iron. A sample of 0.5 ml. of oxalated blood was taken for the iron determination. The Wong's (5) modified method was used to develop the color and the transmittance was read in the Coleman spectrophometer with filter PC-4 at a wave length of 480 $m\mu$ using a reagent blank as the reference solution. The method used was as follows: Transfer with an Ostwald pipette 0.5 ml. of blood into a 50 ml. volumetric flask and introduce 2 ml. of iron-free concentrated sulphuric acid. Whirl the flask to agitate the mixture for 1 or 2 minutes. Add 2 ml. of saturated potassium persulfate solution and

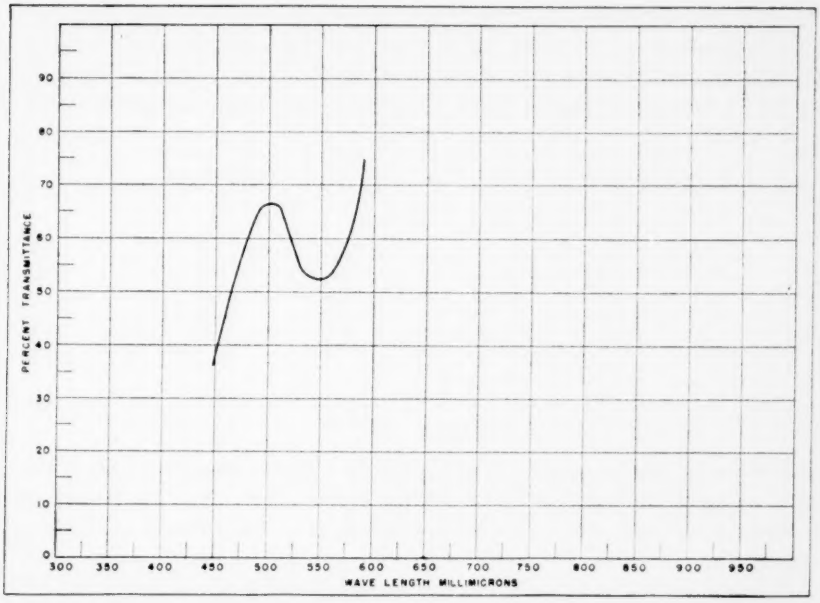

FIG. 2. Spectral-transmittance curve for hemoglobin. Maximum absorption of light at 540 millimierons.

shake. Dilute to about 25 ml. with distilled water and add 2 ml. of 10 per cent sodium tungstate solution. Mix, cool to room temperature under the tap and then dilute to volume with distilled water. Stopper the flask and invert two or three times to effect thorough mixing. Filter through a dry filter paper into a clean, dry receiving vessel. Pipette 20 ml. of the clear filtrate into a large test-tube graduated at 20 ml. and 25 ml. The color was developed by adding 1 ml. of saturated potassium persulfate solution and 4 ml. of 3 N potassium sulfocyanate, KCNS, solution.

The iron standard solution was prepared as follows: Transfer 0.8635

gram of crystallyzed ferrous ammonium sulphate, $FeNH_4(SO_4)_2 \cdot 12H_2O$, to a small beaker and dissolve in about 50 ml. of water. Add 20 ml. of 10 per cent iron-free sulphuric acid. Transfer quantitatively to a liter volumetric flask and dilute to the liter mark with water. One ml. of this solution contains 0.1 mg. Fe. Dilute 10 ml. of this standard solution to 100 ml. with distilled water. One ml. contains 0.01 mg. Fe. The equivalent amount of this standard iron solution was measured in a pipette and poured into the 25 ml. test tube: 0.8 ml. of iron free concentrated sulphuric acid was added, and diluted to the 20 ml. mark with distilled water. Cool to room temperature under the tap, and develop the color as mentioned above, but develop it at the time of reading in the spectrophotometer to avoid fading. The transmittance obtained, for the various iron concentrations using a PC-4 filter, and a wave length of 480 $m\mu$ in the Coleman spectrophotometer, model 11, using the reagent blank as reference solution, was as follows:

IRON CONCENTRATION	TRANSMITTANCE
mg.	%
0.01	83.5
0.02	71.2
0.03	60.0
0.05	42.7
0.07	30.6
0.08	26.0
0.10	19.0

The slope of the standard curve (figure 3) remains constant. Readings from curve give milligrams Fe per 100 ml. blood. The lower transmittance or maximum light absorption was obtained at a wave length of 480 $m\mu$ (figure 4).

Calcium and Phosphorus. The non-oxalated blood was centrifuged, immediately after drawn to avoid hemolysis, for 5 minutes at 2800 r.p.m. in an International clinical centrifuge. The fibrin sealing the plasma was loosened carefully with a wooden rod. The plasma was poured down, or was centrifuged again if necessary, to avoid hemolysis since the red-blood cells of goat's blood are quite minute (diameter = 4.1 microns) and fragile.

Calcium. Calcium in blood serum was determined by the method of Roe and Kahn (4) using a Klett-Summerson photoelectric colorimeter No. 2141, test-tube model, with red filter 66, at a wave length range 640–700 $m\mu$, reading against a reagent blank. The procedure for the blank, unknown, and standard, was as follows:

Unknown. Add 1 volume of serum to 4 volumes of 10% trichloroacetic acid in a small flask and shake well. Pour onto a dry calcium-free filter

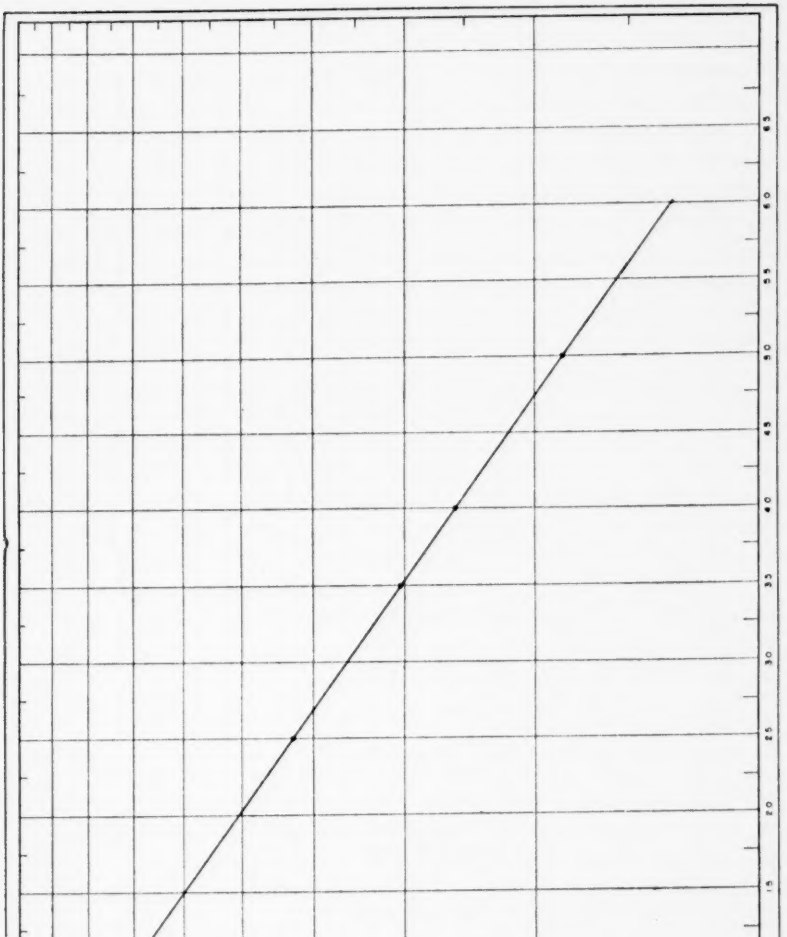

FIG. 3. Iron in blood curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 480 mu, and reagent blank as reference solution. Abscissa represents milligrams of iron as Fe. Readings from curve give milligrams Fe per 100 ml. blood.

paper (Whatman No. 42 or its equivalent) and collect the filtrate in a dry flask. Place 5.0 ml. of the filtrate in a graduated 15 ml. conical centrifuge tube and add 1.0 ml. of 25% sodium hydroxide solution, with mixing by lateral shaking. Allow to stand for 5 minutes, then add 1.0 ml. of 5% trisodium phosphate solution, mix well by lateral shaking, and set aside for an hour. At the end of this time, centrifuge for 2 minutes, and pour off the supernatant fluid, allowing the tube to drain in an inverted position for 2 minutes. Wipe the mouth of the tube dry with a clean cloth. Wash the

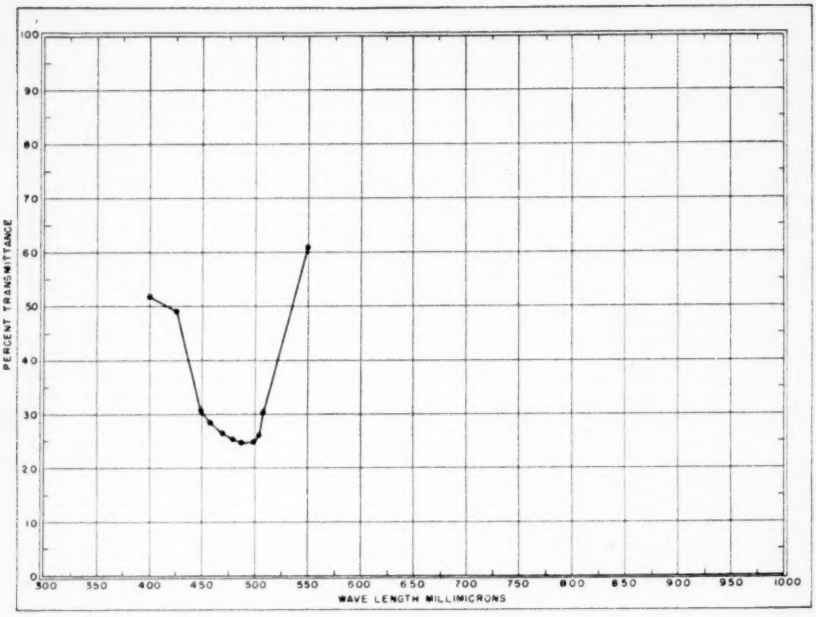

FIG. 4. Spectral-transmittance curve for iron as per method of blood. Maximum absorption of light at a wave length of 480 millimicrons.

precipitate with 5 ml. of alkaline-alcoholic wash reagent (to 10 ml. of amyl alcohol, add 58 ml. of ethyl alcohol, and mix; dilute to 100 ml. with water; add 2 drops of 1 per cent phenolphthalein solution and then, drop by drop, add sufficient 5 per cent sodium hydroxide solution to a distinct pink color; (a few drops should be sufficient); delivered from a pipette with a fine tip, blowing the first portion of wash fluid against the precipitate with such force as to break it up, and using the remainder of wash fluid to rinse down the sides of the centrifuge tube. If necessary use a stirring rod to break up the precipitate. Centrifuge for 2 minutes, pour off the supernatant fluid and allow the tube to drain as before. After draining, wipe the mouth of the

tube dry, and add 2.0 ml. of molybdate reagent (dissolve 25 grams of c.p. ammonium molybdate in 200 ml. water, and pour into a one-liter volumetric flask containing 500 ml. of 10 normal sulfuric acid; dilute to the mark and mix); to dissolve the precipitate and form phosphomolybdate from the phosphate present. After a complete solution of the precipitate, which may be hastened by shaking or stirring, dilute to 10.0 ml. with distilled water, and mix well. Transfer a 5.0 ml. portion from the centrifuge tube to a colorimeter tube and add 0.4 ml. of the aminonaphtholsulfonic acid reagent. Dilute with water to 10.0 ml., mix, and read in the colorimeter after five minutes setting the colorimeter at zero with reagent blank.

Blank. Treat a 5.0 ml. portion of distilled water with 1 ml. of molybdate reagent and 0.4 ml. of aminonaphtholsulfonic acid reagent (weigh 0.125 gram of c.p. 1-amino-2-naphthol-4-sulfonic acid, Eastman Kodak 360, into a 250 ml. beaker containing 50 ml. of 15 per cent sodium bisulfite and 1 ml. of 20 per cent sodium sulfite; shake until dissolved; add a little more of the sodium sulfite solution if necessary to bring the powder into solution; add a little more of the sodium sulfite solution, but an excess should be avoided; keep in a brown bottle away from light; this solution should be prepared fresh every two weeks); and dilute to the 10.0 ml. mark. Set the colorimeter at zero with this reagent blank.

Standard. Treat a 5.0 ml. portion of the standard phosphate solution with 1 ml. of molybdate reagent, add 0.4 ml. of aminonaphtholsulfonic acid reagent, dilute with water to the 10.0 ml. mark, and mix. Read in the colorimeter after 5 minutes. 1 ml. standard phosphate solution = 0.001 milligram P $= \frac{9.67}{5}$ or 1.934 milligrams Ca per 100 ml. blood serum. The readings obtained for the standard curve, after setting instrument at zero with reagent blank, were as follows:

STANDARD P SOLUTION	PHOTOCOLORIMETER READINGS	Ca PER 100 ML. SERUM	SLOPE OF CURVE
mg.		*mg.*	
0.002	76	3.87	0.05092
0.004	155	7.74	0.04994
0.005	192	9.67	0.05036
0.006	228	11.60	0.05088
0.008	310	15.47	0.04990

The slope of the standard curve remains constant. Its average value or factor (figure 5) equals 0.05040. Reading of unknown in photocolorimeter $\times$ 0.05040 equals milligrams of calcium per 100 milliliters blood serum.

Inorganic Phosphorus. Inorganic phosphorus in blood serum was deter-

mined by the method explained in Levinson & MacFate (3) but using a Coleman spectrophotometer, model 11, with filter PC-4 and a wave length of 600 $m\mu$ (figure 7), reading against a reagent blank. The procedure was as follows: Transfer 4 ml. of 10 per cent trichloracetic acid to a small flask and add 1 ml. of the blood serum with shaking. Larger volumes of the serum may be used in the same proportion. Shake well, let stand for 1 to 2 minutes, and filter through a phosphorus-free filter paper. If small quantities of material are used, pour the mixture into a centrifuge tube, centri-

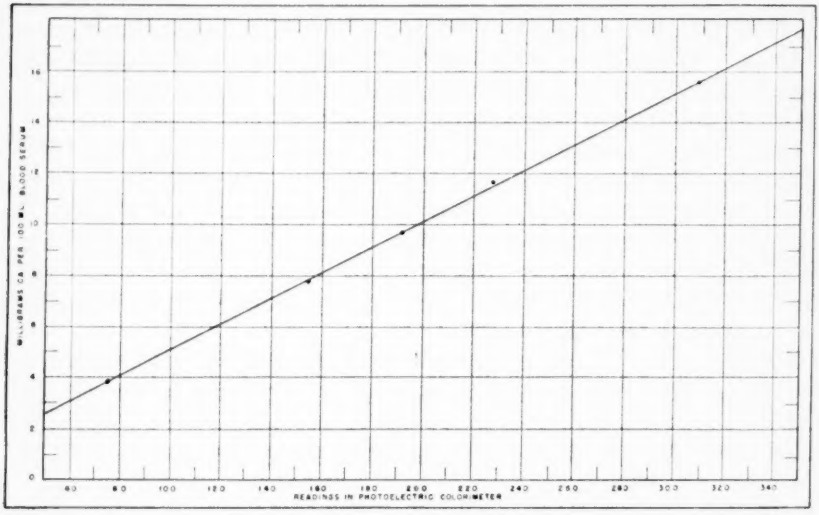

FIG. 5. Calcium in blood serum. Curve obtained in Klett-Summerson photoelectric colorimeter, No. 2141, test tube model, with red filter 66, at a wave length range 640–700 mu, and the instrument at zero with reagent blank. Average slope of curve = 0.0504. Readings of curve $\times 0.0504$ = milligrams of Ca per 100 ml. blood serum.

fuge for a few minutes and filter the clear supernatant fluid through 4.25 centimeter filter paper in a small funnel. Transfer 2 ml. of the filtrate to a 50 ml. Erlenmeyer flask. Add 12 ml. of distilled water, 4 ml. of the molybdic-sulfuric acid reagent A (50 ml. of 10 normal sulphuric acid added to 50 ml. of 7.5 per cent sodium molybdate solution), and 2 ml. of dilute stannous chloride solution (dissolve 10 grams of stannous chloride in 25 ml. of concentrated hydrochloric acid; preserve this stock solution in a brown bottle; dilute 1 ml. of stock solution to 200 ml. with water; preserve in a brown bottle; this dilute solution keeps for 5 days, but is better if prepared fresh each time). Mix well and after 15 minutes read in spectrophotometer.

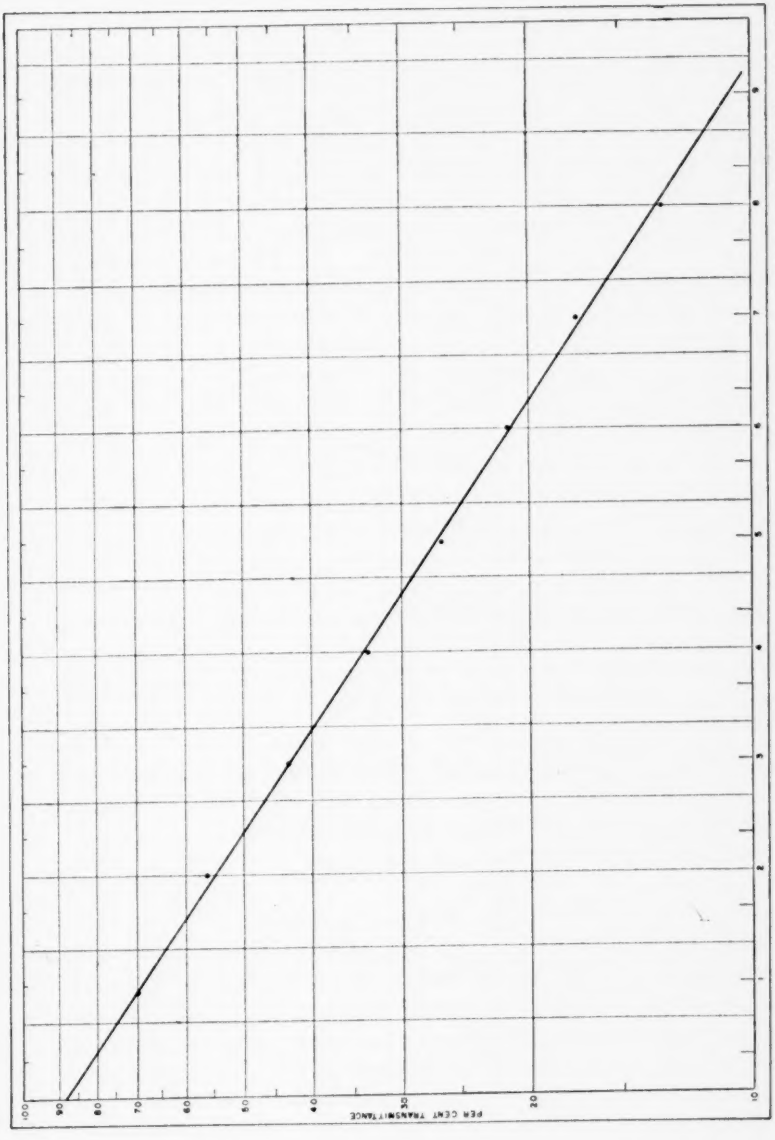

FIG. 6. Inorganic phosphorus in blood serum. Curve obtained in Coleman spectrophotometer, Model 11, with filter PC-4, at a wave length of 600 mu, and the reagent blank as reference solution. Abscissa represents milligrams phosphorus (P) per 100 ml. serum. Slope of standard curve was not found to be constant. Run at least three standards to check slope.

Calibration Curve. Dilute 20 ml. of standard phosphorus solution (1 ml. = 0.01 mg. P) to 25 ml. with distilled water. Dilute to 14 ml. Add 4 ml.

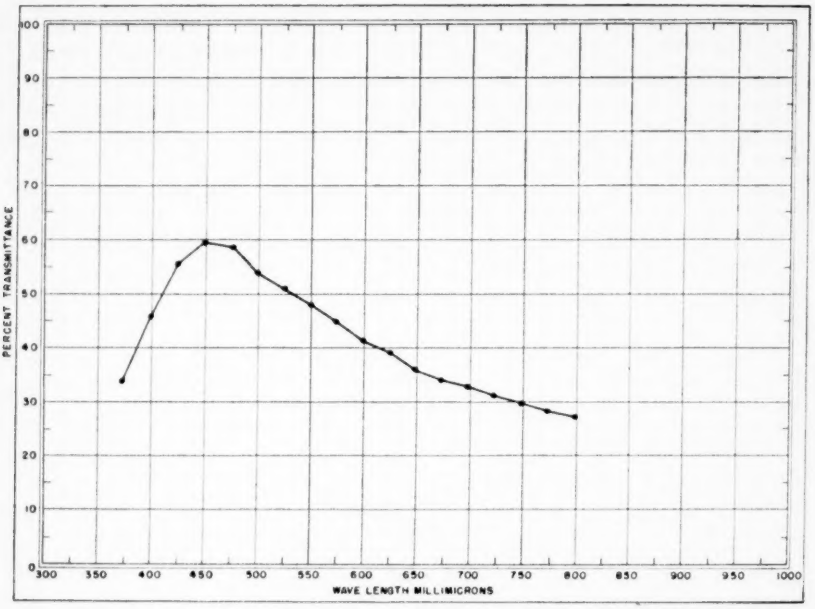

FIG. 7. Special-transmittance curve for phosphorus as per method of blood serum. The PC-4 filter was used for wave lengths, from 350 to 700 mu, and the PC-5 filter, from 700–800 mu.

of the molybdic-sulfuric acid reagent A and 2 ml. of the stannous chloride solution. Read after 15 minutes and plot in semi-log paper.

The following readings were obtained for transmittance:

P-STANDARD	P IN STANDARD	P PER 100 ML. SERUM	TRANSMITTANCE
ml.	*mg.*	*mg.*	%
0.5	0.004	1	70.0
1.0	0.008	2	56.3
1.5	0.012	3	43.7
2.0	0.016	4	34.4
2.5	0.020	5	27.0
3.0	0.024	6	21.9
3.5	0.028	7	17.5
4.0	0.032	8	13.2

Prepare three sets of standards with 1, 5, 8 mgm. P per 100 ml. serum, respectively, simultaneously with the unknown, since it was found that the slope of the standard curve (figure 6) is not constant.

PRESENTATION OF DATA AND DISCUSSION

The monthly weights in pounds per goat and the average monthly weights for the five goats in the treatments: unlimed grass, limed grass, and limed grass plus manganese, are reported for a period of eleven months in table 1. The changes in average weight of the animals in each treatment before and after the pre-gestation period and after the gestation period are condensed in table 2. There was no significant increase in the weight of the goats for the two-month pre-gestation period. However, there was a higher significant decrease of about 8.5 pounds in weight after the six-month gestation period.

The total amount in pounds of the Para-Carib grass mixture and minerals, on the dry basis, eaten by each goat and the average eaten by the five goats in each of the three treatments during the pre-gestation and gestation periods are reported in table 3. The per cent of calcium, phosphorus, manganese, iron and magnesium used for the calculation is that reported previously (1) in part I for the third and fourth grass crops, respectively, in the field at the time of sampling. The total amount of dry grass eaten in the pre-gestation and gestation periods and the total minerals eaten for both periods by the five goats in each treatment are condensed in table 4.

There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments. The goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period the difference for the grass intake between the treatments was not significant.

The average daily grass and mineral intake, in grams per five goats, of about 52 pounds in average weight, is reported in table 5. These data were calculated for a total of 243 days covering the pre-gestation and gestation periods.

The average intake of unlimed grass was about 6 pounds of dry grass per five goats per day and of limed grass was about 7 pounds for animals weighing around 52 pounds.

The goats fed with limed grass received daily about 4 grams more of calcium, 2 grams more of phosphorus, 0.2 grams more of iron, 1 gram more of magnesium, and about 0.2 grams less manganese than the goats fed with unlimed grass. The additional manganese added, per os, to each goat fed with the limed grass, which amounted to 41 milligrams of manganese for the 243 days covering the pre-gestation and gestation periods, was too small to account for an increase in the manganese content of the limed grass.

The average hemoglobin, iron in blood, calcium and phosphorus in blood serum, and blood count per goat in each treatment is reported in table 6. There were no significant differences in the pre-gestation period between the mean red or white-blood cells, the hematocrits, the hemoglobin and iron

TABLE 1

Monthly weights of goats fed with unlimed grass and limed grass with or without extra manganese, for a ten-month period

GROUP	TREATMENT	ANIMAL NUMBER	1944		1945								
			Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
			lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
I	Unlimed grass	81	57.2	56.7	54.8	53.5	51.3	49.5	46.2	48.8	D*	—	—
		90	69.5	68.0	68.8	64.0	64.5	60.2	59.3	54.2	57.7	60.3	58.2
		91	49.5	50.7	49.3	49.0	48.8	46.5	44.8	44.8	43.3	45.2	45.7
		92	48.0	50.0	47.8	45.2	42.7	39.8	37.0	40.0	38.8	39.8	40.3
		94	52.9	54.8	54.0	51.0	52.3	48.8	47.0	48.8	48.0	51.8	51.5
Ave.			55.4	56.0	55.0	52.5	51.9	49.0	46.9	47.3	47.0	49.3	48.9
II	Limed grass	72	39.3	43.3	43.7	41.5	38.7	37.0	35.0	37.3	39.2	39.2	40.5
		80	63.0	64.5	65.8	66.8	63.7	61.3	59.0	63.3	59.7	57.0	53.7
		83	66.8	66.8	67.5	64.5	62.3	57.2	57.3	59.2	44.5	50.2	51.2
		95	52.0	54.2	51.0	49.7	45.5	44.3	39.5	46.5	44.2	45.8	42.0
		97	55.5	55.8	54.8	52.3	50.8	45.5	43.5	48.0	45.8	48.8	48.5
Ave.			55.3	56.9	56.6	55.0	52.1	49.0	46.9	50.9	46.7	48.7	47.2
III	Limed grass & manganese	78	63.5	62.5	63.0	63.2	60.8	58.7	55.8	59.5	57.5	57.5	54.8
		84	49.5	54.2	51.0	50.3	48.3	42.7	42.3	46.7	42.0	45.0	40.0
		87	54.0	54.5	52.0	53.5	52.5	51.8	47.8	51.0	39.8	45.3	48.0
		88	68.2	71.0	65.8	66.7	62.8	61.2	57.3	60.5	60.2	60.0	59.5
		93	46.5	48.2	44.5	45.3	43.3	39.2	37.5	41.5	39.7	43.2	39.5
Ave.			56.3	58.1	55.3	56.0	53.5	50.7	48.1	51.8	47.8	50.2	48.4

* D = died.

TABLE 2

Average weights of the goats, before and after the treatment

TREATMENT	PRE-GESTATION PERIOD		GESTATION PERIOD
	Before	2 months after	6 months after
	lb.	*lb.*	*lb.*
Unlimed grass	55.4	55.0	47.0
Limed grass	55.3	56.6	46.7
Limed grass plus manganese	56.3	55.3	47.8

in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.

The goats were healthy and vigorous at the end of the pre-gestation pe-

TABLE 3

Grass and minerals eaten by goats during pre-gestation and gestation periods (dry basis), pounds

TREATMENT	GOAT NO.	THIRD CROP	FOURTH CROP	TOTAL	CALCIUM (Ca)			PHOSPHORUS (P)			MANGANESE (Mn)			IRON (Fe)			MAGNESIUM (Mg)		
					a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Unlimed grass	81	162	116	278	.33	.34	.67	.45	.28	.73	.03	.04	.07	.03	.01	.04	.30	.24	.54
	90	176	163	339	.35	.47	.82	.48	.39	.87	.03	.04	.07	.04	.02	.06	.32	.33	.65
	91	156	152	308	.31	.44	.75	.43	.36	.79	.02	.04	.06	.03	.02	.05	.29	.31	.60
	92	130	124	254	.26	.36	.62	.36	.30	.66	.02	.03	.05	.03	.02	.05	.24	.26	.50
	94	162	158	320	.33	.46	.79	.45	.38	.83	.03	.04	.07	.03	.02	.05	.30	.33	.63
Total		786	713	1499	1.58	2.07	3.65	2.17	1.71	3.88	.13	.19	.32	.16	.09	.25	1.45	1.47	2.92
Limed grass	72	160	148	308	.54	.50	1.04	.49	.43	.92	.01	.03	.04	.03	.04	.07	.35	.31	.66
	80	195	170	365	.65	.57	1.22	.59	.50	1.09	.02	.03	.05	.03	.04	.07	.43	.35	.78
	83	194	154	348	.65	.52	1.17	.59	.45	1.04	.02	.03	.05	.03	.04	.07	.43	.32	.75
	95	183	132	315	.61	.45	1.06	.56	.39	.95	.02	.02	.04	.03	.03	.06	.41	.28	.69
	97	184	147	331	.62	.50	1.12	.56	.43	.99	.02	.03	.05	.03	.04	.07	.41	.31	.72
Total		916	751	1667	3.07	2.54	5.61	2.79	2.20	4.99	.09	.14	.23	.15	.19	.34	2.03	1.57	3.60
Limed grass plus manganese	78	188	196	384	.63	.66	1.29	.57	.57	1.14	.02	.04	.06	.03	.05	.08	.42	.41	.83
	84	159	139	298	.53	.47	1.00	.49	.41	.90	.01	.03	.04	.03	.03	.06	.35	.29	.64
	87	188	169	357	.63	.57	1.20	.57	.49	1.06	.02	.03	.05	.03	.04	.07	.42	.35	.77
	88	206	189	395	.69	.64	1.33	.63	.55	1.18	.02	.03	.05	.03	.05	.08	.46	.39	.85
	93	154	133	287	.52	.45	.97	.47	.39	.86	.01	.02	.03	.03	.03	.06	.34	.28	.62
Total		895	826	1721	3.00	2.79	5.79	2.73	2.41	5.14	.08	.15	.23	.15	.20	.35	1.99	1.72	3.71

a = third crop, b = fourth crop, c = total.

riod; but were skinny, bony, and weak at the end of the gestation period (see photo). All of them, irrespective of treatment, showed at the end of gestation a reduction in red- and white-blood cells, in hematocrits, in hemoglobin, and in blood iron. However, no change was evidenced in the calcium and phosphorus of the blood serum.

Of the total of fifteen goats; ten aborted, and one died. Each of two goats, fed with limed grass, delivered a weak kid but did not produce milk

TABLE 4

Total dry grass and minerals eaten by the five goats, in each respective treatment, covering the eighth-month pre-gestation and gestation periods

TREATMENT	DRY GRASS EATEN			MINERALS EATEN				
	Pre-gestation period, 2-months	Gestation period, 6-months	Total, 8-months	Ca	P	Mn	Fe	Mg
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Unlimed grass	344	1155	1499	3.65	3.88	0.32	0.25	2.92
Limed grass	419	1258	1677	5.61	4.99	0.23	0.34	3.60
Limed grass plus 203 milligrams of manganese supplied per os to 5 goats	404	1317	1721	5.79	5.14	0.23	0.35	3.71

TABLE 5

Average intake of dry grass in pounds and minerals in grams per day per group of five goats of about 52 pounds each

TREATMENT	DRY GRASS EATEN	MINERALS EATEN				
		Ca	P	Mn	Fe	Mg
	lb.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Unlimed grass	6.17	6.8	7.2	0.60	0.47	5.5
Limed grass	6.90	10.5	9.3	0.43	0.64	6.7
Limed grass plus manganese	7.08	10.8	9.6	0.43	0.65	6.9

even for the newborn. Stylbestrol, at the rate of 5 milligrams per os, which was given to the goats to induce even ovulation, might have been the cause of upsetting their endocrine balance and affecting in general the health of the animal. A marked increase was noticed in the calcium and phosphorus contents of the blood serum one-month after the stylbestrol application (table 7).

Iron combined with hemoglobin and uncombined are reported in table 8. This table contains data for 147 determinations made for iron and hemo-

globin in goat's blood taken monthly for a period of nine months, from each of the fifteen goats in the experiment. The color of the iron was developed by Wong's (5) method, and both, the iron and hemoglobin were determined separately in the spectrophotometer, as already reported. The combined iron in table 8 was calculated by multiplying the hemoglobin by the factor 3.35, as proposed by Wong (5) on the basis that

TABLE 6

Hematological data, iron in blood, calcium and phosphorus in blood serum, for goats in each treatment

TREATMENT	PERIOD	RED BLOOD CELLS PER CU. MM.	WHITE BLOOD CELLS PER CU. MM.	HEMATOCRITS	HEMOGLOBIN	Fe IN BLOOD	Ca IN SERUM	P IN SERUM
		$\times 10^3$		*% vol.*	*gm. %**	*mg. %†*	*mg. %*	*mg. %*
Unlimed grass	Before pregestation	17,626	19,980	31.9	10.5	48.2	11.4	4.9
	End of pregestation	16,894	15,980	30.2	10.1	37.1	12.5	4.0
	End of gestation	15,043	13,475	25.3	8.2	35.4	13.8	4.2
Limed grass	Before pregestation	19,680	19,560	34.2	11.5	51.2	10.6	5.5
	End of pregestation	17,646	18,880	32.5	10.7	39.6	12.2	4.2
	End of gestation	14,662	13,790	23.7	7.9	34.2	14.1	5.2
Limed grass plus manganese	Before pregestation	20,508	16,490	35.7	11.8	51.9	9.9	6.2
	End of pregestation	18,206	15,690	33.0	11.1	40.1	10.2	5.9
	End of gestation	12,624	13,910	22.5	7.6	34.1	13.9	5.2

* Grams hemoglobin per 100 ml. blood.

† Milligrams Fe per 100 ml. blood.

hemoglobin contains 0.0335 per cent iron as Fe. Results in table 8 reveal that considerable of the iron in the blood is not combined with hemoglobin. The uncombined iron varied from 0.1 to 19.9 milligrams per 100 milliliters of blood in 129 blood tests. In 18 tests the iron calculated from the hemoglobin was from 0.79 to 13.0 milligrams per 100 milliliters of blood higher than that found. The low iron content of the blood and the iron deficit in the hemoglobin occurred at regular intervals; the first, at the fifth month period of the experiment; the second, at the ninth month period.

Wong's proposal for calculating hemoglobin from the iron content of the blood does not give, therefore, a true value for hemoglobin, nor does the calculation of blood iron from the hemoglobin content gives a true value for the iron in blood.

A Goat in Its Pen Eating Grass from the Feeding Box
Note poor physical appearance of goat during the gestation period

TABLE 7

Mean calcium and phosphorus contents of blood serum from fifteen female goats before and after stylbestrol application

MINERAL	STYLBESTROL APPLICATION		
	Before	One month after	Two months after
	%	%	%
Calcium, Ca	11.6	17.8	11.6
Phosphorus, P	4.7	6.2	5.7

It has been mentioned that the animals sustained their weight and were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony, and weak at the end of the gestation period. A reduction in red and white blood cells, in hematocrits, in hemoglobin and blood iron,

TABLE 8

Hemoglobin; iron calculated from hemoglobin, iron determined, and uncombined iron, in blood samples taken monthly for a period of nine months from fifteen female goats

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass	81	0	10.20	34.17	46.8	12.63
		1	9.96	33.37	42.7	9.33
		2	9.24	30.95	35.3	4.35
		3	9.00	30.15	35.6	5.45
		4	9.62	32.23	36.8	4.57
		5	9.80	32.83	34.3	1.47
		6	8.32	27.87	33.2	5.33
		7	6.00	20.10	23.3	3.20
		8	—	—	—	—
		9	—	—	—	—
	90	0	11.50	38.53	49.2	10.67
		1	10.20	34.17	44.8	10.63
		2	9.82	32.90	36.2	3.30
		3	9.96	33.37	38.5	5.13
		4	8.30	27.81	35.2	7.39
		5	9.20	30.82	31.4	.58
		6	8.64	28.94	33.2	4.26
		7	6.60	22.11	25.7	3.59
		8	7.44	24.92	33.6	8.68
		9	7.40	24.79	17.5	−7.29
	91	0	9.20	30.82	50.7	19.88
		1	8.66	29.01	43.7	14.69
		2	8.66	29.01	32.5	3.49
		3	10.08	33.77	39.5	5.73
		4	8.90	29.82	38.2	8.38
		5	9.00	30.15	31.2	1.05
		6	8.20	27.47	32.4	4.93
		7	7.38	24.72	29.5	4.78
		8	8.38	28.07	37.5	9.43
		9	8.00	26.80	17.7	−9.10
	92	0	10.50	35.18	47.0	11.82
		1	10.40	34.84	44.8	9.96
		2	11.00	36.85	40.5	3.65
		3	9.98	33.43	38.5	5.07
		4	9.02	30.22	35.3	5.08
		5	9.70	32.50	33.0	.50
		6	8.00	26.80	32.5	5.70
		7	7.78	26.06	28.0	1.94
		8	7.90	26.47	33.8	7.33
		9	7.94	26.60	16.8	−9.80

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
1. Goats receiving unlimed grass —*Continued*	94	0	11.00	36.85	47.5	10.65
		1	11.54	38.66	48.5	9.84
		2	11.54	38.66	40.8	2.14
		3	11.22	37.59	43.3	5.71
		4	9.68	32.43	38.7	6.27
		5	10.80	36.18	32.6	−3.58
		6	10.02	33.57	41.6	8.03
		7	8.16	27.34	—	—
		8	9.20	30.82	36.5	5.68
		9	9.00	30.15	22.8	−7.35
2. Goats receiving limed grass	72	0	12.10	40.54	55.5	14.96
		1	10.80	36.18	43.0	6.82
		2	10.40	34.84	37.7	2.86
		3	11.00	36.85	41.0	4.15
		4	10.16	34.04	42.0	7.96
		5	9.60	32.16	33.6	1.44
		6	9.02	30.22	35.5	5.28
		7	7.00	23.45	28.7	5.25
		8	8.30	27.81	36.4	8.59
		9	7.76	26.00	13.0	−13.00
	80	0	11.80	39.53	49.5	9.97
		1	10.56	35.38	42.5	7.12
		2	11.30	37.86	41.3	3.44
		3	11.38	38.12	42.5	4.38
		4	9.96	33.37	39.7	6.33
		5	10.00	33.50	33.6	.10
		6	9.84	32.96	36.8	3.84
		7	7.46	24.99	40.0	15.01
		8	9.00	30.15	42.3	12.15
		9	8.24	27.60	19.2	−8.40
	83	0	11.00	36.85	54.3	17.45
		1	10.80	36.18	42.7	6.52
		2	10.20	34.17	36.7	2.53
		3	10.80	36.18	41.0	4.82
		4	10.20	34.17	43.4	9.23
		5	10.80	36.18	36.4	.22
		6	9.24	30.95	35.7	4.75
		7	6.80	22.78	24.2	1.42
		8	7.20	24.12	28.0	3.88
		9	7.00	23.45	17.3	−6.15

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg/100 ml.*	*mg./100 ml.*
2. Goats receiving limed grass—*Continued*	95	0	11.00	36.85	49.1	12.25
		1	11.10	37.19	47.0	9.81
		2	10.60	35.51	40.0	4.49
		3	9.92	33.23	39.5	6.27
		4	7.86	26.33	32.7	6.37
		5	9.00	30.15	30.3	.15
		6	7.50	25.13	31.0	5.87
		7	6.80	22.78	28.1	5.32
		8	7.04	23.58	30.5	6.92
		9	6.16	20.64	15.2	−5.44
	97	0	11.40	38.19	47.5	9.31
		1	12.56	42.08	54.0	11.92
		2	10.76	36.05	39.4	3.35
		3	10.08	33.77	39.8	6.03
		4	9.62	32.23	38.7	6.47
		5	9.60	32.16	31.5	− .66
		6	9.50	31.83	34.4	2.57
		7	7.38	24.72	27.8	3.08
		8	—	—	33.8	—
		9	7.90	26.47	18.7	−7.77
3. Goats receiving limed grass plus manganese	78	0	13.20	44.22	61.5	17.28
		1	12.20	40.87	50.5	9.63
		2	12.40	41.54	45.0	3.46
		3	12.18	40.80	46.2	5.40
		4	9.80	32.83	44.0	11.17
		5	12.10	40.54	39.8	− .74
		6	11.06	37.05	41.5	4.45
		7	8.56	28.68	33.8	5.12
		8	8.60	28.81	30.0	1.19
		9	7.92	26.53	18.8	−7.73
	84	0	10.40	34.84	47.6	12.76
		1	12.70	42.55	54.8	12.25
		2	12.20	40.87	43.5	2.63
		3	12.38	41.47	44.8	3.33
		4	8.96	30.02	35.8	5.78
		5	10.40	34.84	35.6	.76
		6	10.20	34.17	40.4	6.23
		7	8.56	28.68	32.8	4.12
		8	7.60	25.46	43.3	17.84
		9	3.60	12.06	5.5	−6.56

TABLE 8—*Continued*

TREATMENT	GOAT NO.	DATE	HEMOGLOBIN	CALCULATED Fe Hb × 3.35	DETERMINED Fe	UNCOMBINED Fe
		mo.	*g/100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
3. Goats receiving limed grass plus manganese—*Continued*	87	0	11.04	36.98	49.0	12.02
		1	10.10	33.84	40.0	6.16
		2	9.36	31.36	36.2	4.84
		3	8.40	28.14	34.6	6.46
		4	9.00	30.15	37.8	7.65
		5	9.40	31.49	30.7	−.79
		6	8.10	27.14	31.7	4.56
		7	6.18	20.70	24.4	3.70
		8	5.86	19.63	25.3	5.67
		9	5.14	17.22	12.5	−4.72
	88	0	12.80	42.88	52.5	9.62
		1	11.80	39.53	47.0	7.47
		2	11.40	38.19	38.5	.31
		3	9.92	33.23	39.6	6.37
		4	10.06	33.70	38.7	5.00
		5	9.40	31.49	34.5	3.01
		6	8.48	28.41	31.4	2.99
		7	7.46	24.99	30.3	5.31
		8	8.60	28.81	37.2	8.39
		9	7.60	25.46	18.6	−6.86
	93	0	11.50	38.53	49.0	10.47
		1	10.90	36.52	43.5	6.98
		2	10.34	34.64	37.4	2.76
		3	9.60	32.16	39.0	6.84
		4	9.80	32.83	38.5	5.67
		5	9.90	33.17	33.6	.43
		6	8.90	29.82	35.1	5.28
		7	7.60	25.46	29.3	3.84
		8	7.20	24.12	34.5	10.38
		9	6.40	21.44	15.2	−6.24

was also reported for the gestation period. It has also been mentioned that two thirds of the goats aborted and that the two goats delivering weak kids produced no milk. Presumably the stylbestrol application upset the endocrine balance and affected the general health of the animals. All indications pointed to malnutrition of the goats during gestation, probably due to an inadequate protein intake.

A composite sample of the grass fed to the animals was not collected during the pre-gestation and gestation periods. The mineral intake for the goats was calculated from the mineral composition of the grass at time of sampling (table 4). The protein intake (table 9) was calculated similarly.

During the pre-gestation period the goats were fed exclusively with grass from the third crop (1) that received a nitrogen application at the rate of 500 pounds of ammonium sulphate per acre. The protein content of this grass at time of sampling, or 36 days after nitrogen application, was 11.2 per cent.

During the gestation period the goats were fed 72 days with high protein grass from the third crop and 110 days with a low protein grass from the fourth crop that received no nitrogen fertilization.

The daily intake of protein per goat for the pre-gestation period was estimated to be 0.143 pounds or about 65 grams, and for the gestation period was .089 pounds or about 40 grams (table 9). The goats ate per day about the same amount of grass in the pre-gestation and gestation periods, but the

TABLE 9

Estimated protein intake per goat per day during the pre-gestation and gestation periods

PERIOD	TIME OF PERIOD	AMOUNT OF GRASS EATEN PER GOAT PER		PROTEIN CONTENT OF GRASS AT TIME OF SAMPLING	ESTIMATED AMOUNT OF PROTEIN EATEN PER GOAT PER DAY
		Period	Day		
	days	*lbs.*	*lbs.*	*%*	*lb.*
Pre-gestation	61	77.8	1.28	11.2	0.143
Gestation	72	98.4	1.37	11.2	0.153
	110	150.3	1.37	3.5	0.048
	182	248.7	1.37		0.089

daily intake of protein was 25 grams less in the gestation period. This protein deficiency might have been the cause for the malnutrition of the goats during the gestation period.

CONCLUSION

Female goats kept in their pens, and fed exclusively with a mixture of unlimed and limed Para or "Malojillo" grass (*Panicum purpurascens*) and Carib or "Malojilla" grass (*Eriochloa polystachya*), suffered malnutrition effects during the six-month gestation period. The low normal protein content of the grass mixture, around 3.5 per cent, was probable the main factor involved. Malnutrition was unnoticed during the two-month pre-gestation period because, due to adequate and timely nitrogen fertilization the protein content of the grass mixture was raised to a higher level, around 11 per cent.

Nutrition studies involving mineral in animal blood require that the animal be fed with an adequate protein level during the whole experimental period.

SUMMARY

This paper reports spectrophotometric methods and transmittance-concentration curves for hemoglobin and iron in blood and phosphorus in blood serum and a photocolorimeter method and curve for calcium in blood serum.

Fifteen cross-bred virgin female goats were randomized in groups of five and fed according to three treatments: unlimed grass, limed grass, and limed grass plus manganese per os. Amounts of grass fed to each animal and their monthly weights were recorded for a one-month prefeeding period, a two month pre-gestation period, and a six-month gestation period. Blood samples were also drawn monthly from each animal for determination of hemoglobin, iron, hematocrits, red blood cells and white blood cells in blood, and calcium and phosphorus in blood serum.

The data are reported in nine tables. The important results are as follows:

1. There was no significant increase in the weight of the goats for the two-month pre-gestation period; however, there was a highly significant decrease in weight after the six-month gestation period.
2. There were significant differences for the pre-gestation period between the grass intake of the goats under the three treatments; the goats fed with unlimed grass ate less than those fed with limed grass. However, for the gestation period, the differences for the grass intake between the treatments was not significant.
3. The average intake of dry grass in pounds, and mineral in grams per day per group of five goats, covering the 243 days of the pre-gestation and gestation periods, are reported in table 5.
4. There were no significant differences in the pre-gestation between the mean red or white blood cells, the hematocrits, the hemoglobin and iron in the blood, and the calcium and phosphorus in the serum of the goats in the three treatments.
5. The goats were healthy and vigorous at the end of the pre-gestation period; but were skinny, bony and weak at the end of the gestation period (see photo).
6. The goats at the end of gestation, showed a reduction in red and white blood cells, in hematocrits, in hemoglobin, and in blood iron. However, no change was evidenced in the calcium and phosphorus of the blood serum.
7. Of the total of fifteen goats; ten aborted, and one died. Each of two goats delivered a weak kid, but produced no milk.
8. Stylbestrol, applied at the rate of 5 milligrams per os to each goat, to induce even ovulation, might have been the cause of upsetting the endocrine balance and affecting the health of the animal. A

marked increase was noticed in the calcium and phosphorus contents (table 7) of the blood serum after the stylbestrol applications.

9. Not all of the iron present in the blood is combined with hemoglobin (table 8).
10. The estimated protein intake per goat during the pre-gestation period was calculated to be about 65 grams per day (table 9), while that during the gestation period was about 40 grams per day. This protein deficit in the gestation period might have been an important factor causing malnutrition in the goats.

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LABORATORY RECOMMENDATION OF LIME TO AN ACID SOIL CHECKS WITH EXPECTED pH CHANGES

ALFONSO RIERA

INTRODUCTION

The agricultural value of about one million acres of acid soils in the humid area of Puerto Rico may be improved with the application of lime. The textures of these soils vary from the sands to the clays. Their organic matter has been reported (1) to vary from 1.0 per cent in the sandy soils to 51.5 per cent in the mucks. The buffer capacity of these soils is variable. A laboratory method for determining the lime requirement, which will be applicable to field conditions, is, therefore, of paramount importance.

Dr. B. G. Capó, Head of the Department of Agronomy and Horticulture, worked as a Soil Chemist in the Department of Soils from 1936 to 1942. While he was working in pot studies with soil-sand mixtures, using Hegari sorghum as a plant index to determine the available major nutrients in the soils, he adopted the following lime-requirement method to bring the acid soils to a convenient pH value.

LABORATORY METHOD

The air-dried soil samples are ground to pass a 1 mm. sieve. Calcium carbonate c.p. Baker Analized, is used as the lime source. Five portions of 20 grams of soil are weighed and placed in each of five 250 ml. beakers. The amounts of calcium carbonate added respectively, to each beaker, are 20, 40, 60, 80 and 100 milligrams corresponding to 1, 2, 3, 4 and 5 tons of calcium carbonate per acre. One hundred and fifty cubic centimeters of distilled water are added to each beaker. The mixture is stirred for 3 hours in a Ross-Kershaw apparatus, described as #9235 of Arthur H. Thomas catalog. The pH values of the supernatant liquids are determined and plotted on coordinate paper against the tons of calcium carbonate. The amount of limestone necessary to bring the pH of the supernatant liquid to the desired pH value is estimated from the corresponding curve.

FIELD RESULTS

"Fajardo clay" is an acid soil type derived from ashy shale. This soil type is well distributed in the terraces of the Experiment Station farm. Soil samples, at six-inch depths were taken on June 1943 from each of nine plots of "Fajardo clay" for the lime requirement tests. The amount of limestone necessary to bring the soil of each plot up to pH 6.5 was cal-

culated from the corresponding pH-lime requirement curve as described above.

The required amount of commercial ground limestone was applied to each of these plots and "malojillo" (Para) grass was planted on them on July 1943. On October 1944, soil samples were taken again at each of these plots, for pH determinations with the results that appear on table 1.

TABLE 1

Values of pH before and after liming

PLOT NUMBER	pH VALUE BEFORE LIMING	CALCIUM CARBONATE TO RAISE UP TO pH 6.5	pH VALUE 15 MONTHS AFTER LIMING
		tons/acre	
1	4.4	10.0	6.2
4	4.4	10.0	6.3
5	4.1	8.0	6.4
8	4.6	10.0	6.6
9	4.7	10.0	6.7
12	4.9	10.0	6.6
13	5.5	8.0	6.9
18	4.2	12.0	5.2
20	4.6	10.0	6.1

The difference between the mean pH values does not differ statistically from the desired value 6.5.

SUMMARY

A laboratory method for lime requirement in soils is presented. Lime was applied to the acid soil of nine plots of a field experiment at the rate found by this method. The pH changes fifteen months later did not differ statistically from the ones expected.

RESUMEN

Se expone aqui un método de laboratorio relacionado con la cal que necesitan los terrenos. Se aplicó cal a los terrenos ácidos de nueve parcelas que constituyeron el campo de un experimento, en la proporción determinada por dicho método. Los cambios pH, quince meses después, no se diferenciaron estadisticamente, de aquellos que se habían esperado.

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LACK OF RESPONSE OF SUGARCANE TO APPLICATIONS OF PHOSPHORUS IN PUERTO RICO

J. A. BONNET, B. G. CAPÓ AND A. RIERA

INTRODUCTION

In the last decade, 1934–1944, sugarcane, the most important economic crop of Puerto Rico, has occupied an average of 303,678 acres. The peak of total production was reached with the 1941–1942 crop which amounted to 10,010,132 tons of cane and yielded 1,147,589 tons of sugar. The Association of Sugar Producers of Puerto Rico reports that in 1941, from January to December, 141,000 tons of fertilizer raw materials were imported—80,000 tons ammonium sulphate, 36,000 tons superphosphate and 25,000 tons potash salts—from which about 80 per cent or 112,800 tons were used for sugarcane. The normal application for fall and spring plantings of sugarcane in Puerto Rico is six bags of 200 pounds each, per acre; and for ratoons, 4 bags per acre. Except for certain sections of the arid region of the South coast of the Island where ammonium sulphate is applied only, a complete fertilizer is used as a general practice. From July 1941 to June 1942, both inclusive, 92,975 tons of complete fertilizers (5) were applied to sugarcane in Puerto Rico. The available phosphoric acid in those fertilizers varied between four and ten per cent and added up to 5,658 tons P_2O_5. At the estimated price of $28.00 per ton of superphosphate containing 20 per cent available phosphoric acid, the consumption of phosphate fertilizer for the 1941–42 crop amounted to $792,120. The investment in phosphorus for sugarcane for the last 24 years (1920–1944) is estimated to be around ten million dollars.

The official fertilizer formulae for sugarcane approved by the War Production Board for manufacture in 1944–45, on the basis of NH_3,P_2O_5 and K_2O, are: 10–6–9, 12–4–9, 14–6–5, 14–6–8, 14–3–8, 14–0–7, 14–0–11. The demand for the formulae containing no phosphorus, however, has been practically negligible.

It is of economic importance, therefore, to determine if the continuous addition of phosphorus to the fertilizer is necessary for the sugarcane crop. It is also of importance to know if the placement of the fertilizer has any effect upon the response of the cane plant to applications of phosphorus.

EXPERIMENTAL WORK

A number of field experiments has been conducted in the past few years in which the need of phosphorus applications to maintain or increase

sugar yields has been under study. In these experiments, phosphoric acid fertilizers have been tried in different amounts, the rate of the phosphoric acid applications varying from zero to a maximum of four hundred P_2O_5 per acre. The actual rates of application as well as the mean cane yields obtained in these experiments are presented in tables 1 and 2.

The experiments were performed with several sugarcane varieties and they were established in the following 14 soil types (4) representing 84,224 acres of the most important sugarcane producing soils: "Toa silty clay loam", "Toa silty clay", "Toa clay", "Coloso silt loam", "Coloso silty clay loam", "Coloso silty clay", "Aguirre clay", "Vayas clay", "Vega Baja silty clay", "Mercedita clay", "Mabí clay", "Moca loam", "Vega Alta clay loam" and "Cataño sandy loam". The description of the soil series to which these soil types belong is as follows:

Toa. Series of the well-drained soils of the river flood plains in the humid area, derived from materials washed from the limestone and tuffaceous hills. They are neutral or slightly acid in reaction and are high in bases and plant nutrients. They are friable, brown soils from the surface to a depth of below four feet. The productivity rating of the loam to clay types is 1, i.e., they are rated among the best soils.

Coloso. Series of the poorly drained soils of the river flood plains in the humid area, derived from neutral, fine textured materials of volcanic rocks and limestone. They are poorly drained associates of the "Toa" series. They are deep, stone free, highly fertile, neutral, and plastic. Most areas have a heavy texture, a high water table, a dark surface soil, and a mottled-gray, bluish-gray, and rust-brown subsoil. The productivity of the heavy types is rated between 1 and 2.

Aguirre. Series of the poorly drained soils of the river flood plains in the arid area, that occupy areas that are transitional in character between the soils of the well drained river flood plains or alluvial fans, and the poorly drained soils of the coastal lowlands. In a cultivated field, "Aguirre clay" has a 10 or 12 inch very dark, grayish-brown and gray plastic sticky clay that continues to a depth ranging from 30 to 36 inches. The upper part of the substratum is a mottled-gray, rust-brown, and yellowish-brown, medium plastic, wet silty clay, that in places contains some medium-sized gravel. At a depth ranging from 5 to 6 feet is the substratum of bluish-gray, plastic, sticky wet clay. This layer continues to considerable depths and has characteristics of estuarine deposits. Nearly all of the layers in the profile contain free lime; many areas contain salts, chiefly sodium carbonate, that limit crop production. Areas that contain less than 0.2 per cent of salts within the first four feet are used under irrigation for the production of sugarcane. It has a productivity rating of 2. The

TABLE 1

Percentage increases in cane yields obtained when phosphoric acid applications were made

CROP NO.	SOIL TYPE AND LOCATION	CANE VARIETY	KIND OF CROP AND YEAR	FERTILIZERS APPLIED PER ACRE			CANE YIELDS PER ACRE	INCREASE IN YIELD
				NH_3	P_2O_5	K_2O		
				cwts.	*cwts.*	*cwts.*	*tons*	*per cent*
1	Coloso silty clay, Toa Baja	BH-10(12)	Plant Cane	4	0	4	69.3	1.3
			1937–1939	4	4	4	70.2	
2	" "	BH-10(12)	First Ratoon	4	0	4	50.7	−7.9
			1939–40	4	4	4	46.7	
3	" "	BH-10(12)	Second Ratoon	4	0	4	37.6	8.5
			1940–41	4	4	4	40.8	
			Average of 3 crops	4	0	4	52.5	0.0
				4	4	4	52.6	
4	Coloso silt loam Toa Baja	BH-10(12)	Plant Cane	4	0	4	68.4	4.1
			1938–40	4	4	4	71.2	
5	" "	BH-10(12)	First Ratoon	4	0	4	49.8	−5.8
			1940–41	4	4	4	46.9	
			Average of 2 crops	4	0	4	59.1	0.0
				4	4	4	59.1	
6	" "	POJ-2878	Plant Cane	4	0	4	65.3	−0.5
			1938–40	4	4	4	65.0	
7	" "	POJ-2878	First Ratoon	4	0	4	62.4	−3.4
			1940–41	4	4	4	60.3	
			Average of 2 crops	4	0	4	63.9	−1.9
				4	4	4	62.7	
8	" "	M-28	Plant Cane	4	0	4	66.7	0.6
			1938–40	4	4	4	67.1	
9	" "	M-28	First Ratoon	4	0	4	45.5	−8.6
			1940–41	4	4	4	41.6	
			Average of 2 crops	4	0	4	56.1	−3.0
				4	4	4	54.4	
10	" "	M-275	Plant Cane	4	0	4	67.2	1.3
			1938–40	4	4	4	68.1	

TABLE 1—*Continued*

CROP NO.	SOIL TYPE AND LOCATION	CANE VARIETY	KIND OF CROP AND YEAR	FERTILIZERS APPLIED PER ACRE			CANE YIELDS PER ACRE	INCREASE IN YIELD
				NH_3	P_2O_5	K_2O		
				cwts.	*cwts.*	*cwts.*	*tons*	*per cent*
11	Coloso silt loam Toa Baja	M-275	First Ratoon	4	0	4	58.4	4.3
			1940–41	4	4	4	60.9	
			Average of 2	4	0	4	62.8	2.7
			crops	4	4	4	64.5	
12	Aguirre clay, Salinas	BH-10(12)	Plant Cane	4.5	0	4	45.1	−8.0
			1940–42	4.5	3	4	41.5	
13	" "	POJ-2878	Plant Cane	4.5	0	4	42.8	5.1
			1940–42	4.5	3	4	45.0	
14	Mabi clay, Juncos	POJ-2878	Plant Cane	4.5	0	4	75.4	4.8
			1940–42	4.5	3	4	79.0	
15	Mercedita clay, Ponce	BH-10(12)	Plant Cane	4.5	0	4	97.0	−1.9
			1940–42	4.5	3	4	95.2	
16	" "	POJ-2878	Plant Cane	4.5	0	4	108.8	−6.2
			1940–42	4.5	3	4	102.1	
17	Toa silty clay, Manatí	M-275	Plant Cane	2.325	0	2.325	94.9	10.6
			1941–43	2.325	1.55	2.325	105.0	
18	Toa silty clay loam, Manatí	BH-10(12)	Plant Cane	3	0	3	74.2	−0.7
			1942–43	3	2	3	73.7	
19	Coloso silt loam, Fajardo	BH-10(12)	Plant Cane	4	0	4	89.8	−8.7
			1937–39	4	4	4	82.0	
20	" "	BH-10(12)	First Ratoon	4	0	4	48.4	11.4
			1939–40	4	4	4	53.9	
			Average of 2	4	0	4	69.1	−1.6
			crops	4	4	4	68.0	
21	Moca loam, Toa Baja	POJ-2878	Plant Cane	3	0	3	40.4	7.7
			1939–40	3	3	3	43.5	
22	Coloso silty clay loam, Naguabo	BH-10(12)	First Ratoon	3	0	3	81.9	−2.4
			1937–39	3	3	3	79.9	
23	Cataño sandy loam, Añasco	POJ-2878	Plant Cane	0.9	0	0.9	47.4	5.3
			1937–38	0.0	0.9	0.9	49.9	
24	Toa clay, Hormigueros	POJ-2878	Plant Cane	0.9	0	0.9	36.9	1.4
			1937–38	0.9	0.9	0.9	37.4	

land is difficult to plow and cultivate because when wet it is plastic and sticky, and when dry it is hard and cloddy.

Vayas. Series that occupy the more poorly drained level areas along

TABLE 2

Percentage increases in cane yields obtained with increases in phosphoric acid applications above the minimum 0.20 cwt. P_2O_5 per acre applications

TREATMENTS			POJ-2878, VEGA ALTA CLAY LOAM, RÍO PIEDRAS, FIRST RATOON, 12 MONTHS. 1943-44		BH-10(12), VAYAS CLAY, SANTA RITA			
					First ratoon, 12 months. 1942-43		New planting of plant cane, 10 months. 1943-44	
NH_3	P_2O_5	K_2O	Yield	Per cent increase	Yield	Increase	Yie d	Increase
cwt./A.	*cwt./A.*	*cwt./A.*	*tons/A.*		*tons/A.*	%	*tons/A.*	%
1.25	0.20	0.90	39.9		33.8		24.4	
1.25	0.80	0.90	38.8	−2.8	30.0	−11.2	24.1	−1.2
1.25	0.20	1.80	37.0		30.5		22.5	
1.25	0.80	1.80	39.1	5.7	29.9	−2.0	23.3	3.6
1.25	0.20	2.70	39.6		27.0		25.5	
1.25	0.80	2.70	40.6	2.5	31.4	16.3	24.0	−5.9
2.00	0.20	0.90	36.9		29.9		21.0	
2.00	0.80	0.90	37.0	0.3	32.5	8.7	25.3	20.5
2.00	0.20	1.80	38.8		29.3		21.0	
2.00	0.80	1.80	39.3	1.3	32.8	11.9	26.9	28.1
2.00	0.20	2.70	38.0		30.1		23.1	
2.00	0.80	2.70	37.9	−0.3	29.1	−3.3	26.9	16.5
2.75	0.20	0.90	37.9		32.3		24.4	
2.75	0.80	0.90	38.6	1.8	30.6	−5.3	22.1	−9.4
2.75	0.20	1.80	41.3		34.3		26.6	
2.75	0.80	1.80	37.1	−10.2	27.5	−19.8	20.5	−22.9
2.75	0.20	2.70	36.1		32.8		26.8	
2.75	0.80	2.70	37.9	5.0	29.9	−8.8	23.6	−11.9
3.50	0.20	0.90	37.9		29.9		23.5	
3.50	0.80	0.90	34.6	−8.7	33.3	11.4	28.1	19.6
3.50	0.20	1.80	40.0		29.5		26.3	
3.50	0.80	1.80	35.5	−11.3	31.4	6.4	24.4	−7.2
3.50	0.20	2.70	37.6		32.9		26.5	
3.50	0.80	2.70	38.5	2.4	30.3	−7.9	27.5	3.8
Average with 0.20 cwt. P_2O_5 p/A			38.4		31.0		24.3	
Average with 0.80 cwt. P_2O_5 p/A			37.9	−1.3	30.7	−1.0	24.7	1.6

the river flood plains in the semiarid south coast section. "Vayas clay" has a brown or dark-brown clay in the surface and a mottled-gray or rust-brown and yellowish-brown layer beginning at a depth of about 30 inches and continuing to considerable depths. The average upper limit of the

water table is about 30 inches. Nonalkali areas have a productivity rating of 1. When wet it is plastic, and when it dries large cracks appear on the surface.

Vega Baja. Series of the poorly drained soils of the river flood plains in the humid region. They occupy bordering or intergrade areas between the alluvial soils and the coastal plain soils. They are slightly above normal overflow, but during exceptionally high water are flooded. The surface soil of the silty-clay type, to a depth of eight or ten inches, is acid, friable and granular. It has a light-brown or grayish-brown color. This layer changes abruptly to a plastic, medium compact, mottled, yellowish-brown, gray and red silty clay or clay subsoil, which continues to considerable depth, and becomes more definitely mottled and more acid with depth. Its productivity is rated between 2 and 3.

Mercedita. Series of the inner plain soils in the arid region. The 12 inch surface soils of Mercedita clay consist of brown or dark-grayish-brown, granular calcareous clay, which is very plastic and waxy when wet. The subsoil is yellowish-brown or light olive-brown, medium compact, plastic calcareous clay, ranging from 10 to 15 inches in thickness. Below this layer is a very limy, friable, light-yellow silty material and soft limestone, which continues below a depth of 5 feet. Some areas contain harmful quantities of salts. Many areas are affected with lime chlorosis Its productivity is rated at 2.

Mabí. Series of the inner plain soils in the humid region. "Mabí clay" occurs on long low gentle slopes, in close association with the "Múcara" and related brown, shallow soils of the uplands. It is derived from tuffaceous material, partly residual, and in part colluvial and alluvial. It has a grayish-brown, neutral, plastic heavy, clay surface soil, about eight or ten inches thick, which has good tilth when properly plowed and cultivated. The subsoil is a yellowish-brown, plastic sticky, neutral, heavy clay, streaked with rust-brown and gray material. This layer gradually changes, at a depth ranging from 30 to 40 inches, to friable brown and yellowish-brown silty clay loam that crumbles readily between the fingers. The material grades into desintegrated tuffaceous rock material at a depth ranging from four to 12 feet. Small fine white specks of the rock material occur in all layers. Its productivity is rated at 3.

Moca. Series of the inner plain soils in the humid area. "Moca loam" occurs on low sloping areas near the limestone hills. It has eight or ten-inch, acid, brown or dark-brown friable loam, which abruptly changes to heavy plastic, sticky, acid silty clay, or clay that is mottled red, gray, and brown. This layer continues downward for many feet. All layers contain a few rounded pieces of gravel or small rocks. Its productivity is rated at 6.

Vega Alta. Series of the friable soils of the coastal plains in the humid

region. "Vega Alta clay loam" has a friable, brown or light-brownish-gray, acid surface, about eight inches thick, underlain by a reddish-brown, heavy, slightly plastic clay layer about 10 or 12 inches thick. This layer rests on more compact, mottled, brown, red, and gray clay, which continues to great depths before limestone is reached. Its productivity is rated at 5.

Cataño. Series belonging to the soils of the coastal lowlands in the humid region. It occurs as a narrow strip paralleling the sea, a short distance inland. It has a 10 or 12 inch surface soil of grayish-brown or dark-grayish-brown, loose noncoherent loamy sand, alkaline in reaction. This layer is underlain by a lighter colored and lighter textured calcareous subsoil about two feet thick. The substratum, to a depth ranging from 10 to 15 feet, is loose, friable sand. Its productivity is rated at 6.

The mean contents of phosphoric acid available in 1% citric acid, for various samples of soil types corresponding to the above soil series have been reported elsewhere (2.3) and are as follows:

SOIL SERIES	SAMPLES ANALYZED	AVAILABLE P_2O_5
Toa	21[1]	.012
Coloso	18	.015
Aguirre	1	.041
Vayas	1	.072
Vega Baja	4	.013
Mercedita	1	.001
Mabi	11	.005
Moca	3	.003
Vega Alta	5	.008
Cataño	8	.017

The mean contents of total phosphoric acid of 7 samples of the Toa series and of 6 samples of the Coloso series were found (2) to be .113 and .154 per cent, respectively.

In general, the soil series of the arid region of Puerto Rico (3) contain about twice as much available phosphoric acid, soluble in 1% citric acid, than the soil series of the humid region. The mean difference is significant at the 1% point. The figures are as follows:

PER CENT AVAILABLE P_2O_5 IN SOIL SERIES OF		
Humid Area	Arid Area	Mean Difference
.0079 ± .0007	.0173 ± .0030	.0094 ± .002

[1] Highly significant.

EXPERIMENTAL RESULTS

Table 1 shows the yields of cane, in tons per acre, obtained in twenty-four crops of various sugarcane field experiments, both without and with a heavy application of phosphoric acid. Heavy applications of nitrogen and potash were added in all cases to insure the maximum possible responses from the phosphoric acid applications.

The first eighteen crops are from experiments performed in cooperation between the Soils and Agronomy Departments of the Agricultural Experiment Station of the University of Puerto Rico. Crops number 19 and 20 belong to an experiment carried out by Mr. Juan E. Veve, while at the Central Fajardo Experiment Station; crop no. 21 is from an experiment performed by Mr. Fernando Chardón at Central Constancia; crop no. 22 is from an experiment carried out by Mr. H. A. Nadler, Jr. of Eastern Sugar Associates; and crops nos. 23 and 24 were reported (1) by the Puerto Rico (Mayaguez) Agricultural Experiment Station.

Table 2 presents data relative to three crops of two long-time sugarcane field tests which are being performed by the Soils Department of the Agricultural Experiment Station of the University of Puerto Rico at Río Piedras and Ensenada.

The mean cane yield differences obtained with the various phosphoric acid applications in each of the experiments described in tables 1 and 2 were not statistically significant. This indicates that the sugarcane yields were not affected by the phosphoric acid applications, even in the case of crop number 3, which was the third crop in succession in which a heavy application of phosphoric acid was tested against no addition of this substance. That is, the phosphoric acid which this soil contained at the beginning of this test sufficed at least for maximum cane yield production of a plant cane and two ratoon crops. Similar statements may be made for the two consecutive crops, number 4 to 11 and 19 to 20.

The lack of response of the sugarcane crop to the phosphoric acid applications in these experiments may have been due to one of two reasons:

1. The soils were in condition to supply the crops with enough phosphoric acid as to render unnecessary and superfluous any further applications of the substance.
2. The phosphoric acid, applied as superphosphate on top of the soil in some of the experiments described in table 1, was not able to penetrate to the root zone and did not exert its possible beneficial action on the crop.

If the first of the two reasons advanced above is applicable, then it must be admitted that sugarcane behaves differently from other crops such as native red beans, eggplant, corn, cucumbers, and sudan grass, all of which have responded markedly to phosphoric acid applications in several ex-

periments performed on soil types similar to those in which the sugarcane has not responded to those applications. The long-time field experiments established at Río Piedras and Ensenada will be continued for a long number of years to determine how long will the minimum 20 pounds P_2O_5 per acre under test be able to maintain crop yields at the same levels as those obtained with the 80 pounds P_2O_5 per acre applications.

TABLE 3

Effect on the sugar cane yield of the method of applying the fertilizer

NUMBER	TREATMENT	F-1017 ON "VEGA BAJA SILTY CLAY," RÍO PIEDRAS. AUGUST 1940 TO MAY 1944			
	Method of applying the fertilizer, (300 lbs. NH_3, 100 lbs. P_2O_5 and 200 lbs. K_2O, per acre per crop)	Plant cane	First ratoon	Second Ratoon	Total for 3 crops
		Tons cane per acre			
1	Phosphate for plant cane and two ratoons mixed with the soil in the furrow before planting; ammonia and potash applied to each crop on two 3-inch deep furrows at the sides of each row one month after last replanting	66.2	50.7	31.7	148.6
2	Phosphate for plant cane and two ratoons mixed with the soil in the furrow before planting, ammonia and potash on top soil for each crop, one month after last replanting	67.0	53.2	30.5	150.7
3	Complete fertilizer applied to each crop on 3-inch deep furrows at the sides of each row, one month after last replanting	69.6	53.0	31.5	154.1
4	Check—Complete fertilizer applied to each crop, on top of the soil, one month after last replanting	68.4	53.8	34.0	156.2

To test the possibility of the second of the two reasons presented above, a fertilizer placement experiment was performed on a "Vega Baja silty clay" field very near to fields where heavy responses to the phosphoric acid applications had been observed with sudan grass and red beans.

In this experiment, different ways of applying the phosphoric acid were tested. The rates of application, yields obtained and other details are presented in table 3.

The statistical analysis of the results obtained in this experiment indicated that none of the differences between the mean yields corresponding to the various ways of applying the fertilizer was significant. In this case, therefore, the second reason is not applicable.

In view of the above results, it may be concluded for the time being that maximum sugar cane crops may be raised in the coastal plains of Puerto Rico without the application of any phosphoric acid for at least one crop cycle, that is, a plant cane and 2 ratoon crops. The results of the long-time tests under way at present will indicate in the near future whether this period may be lengthened and, if so, by how much.

The above results do not corroborate the statement which appeared in page 84 of the 1927–28 annual report of the Insular Experiment Station of Puerto Rico to the effect that an application of "phosphoric acid in excess of sixty pounds per "cuerda" (0.9712 acre) lowers the gain in yields of sugarcane". As the above data indicate, phosphoric acid applications, up to a maximum limit of four hundred pounds P_2O_5 per acre, have not affected, either for better or worse, the cane yields in the lowlands of Puerto Rico in experiments lasting a maximum of three and a half years. It is believed that the harmful effect of the heavy applications of phosphoric acid, was rather due to the fact that in the treatments involving such phosphoric acid applications the nitrogen level was lower than where smaller applications of phosphoric acid were made.

SUMMARY

1. The yearly investment in phosphoric acid as a fertilizer for sugarcane in Puerto Rico is about $800,000.

2. Phosphoric acid applications have not exerted any effect, either beneficial or detrimental, on the cane yields of several successive sugarcane crops grown on the coastal plains of Puerto Rico.

3. Sugarcane may be raised for several years in the coastal plains of Puerto Rico without any phosphoric acid applications and with no appreciable reduction in sugarcane yields.

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